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PROCEEDINGS
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*AEROBIC FORMATION OF FUMARIC ACID IN THE MOLD RHIZOPUS NIGRICANS: SYNTHESIS BY DIRECT C_2 CONDENSATION**

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Communicated by S. A. Waksman, October 21, 1949

Recent studies[1] have demonstrated that fumaric acid formation from glucose by *Rhizopus nigricans* No. 45 involves at least two mechanisms, one of which is aerobic, the other anaerobic. The latter involves a bulk fixation of CO_2 via oxalacetate, in confirmation of the reaction qualitatively demonstrated in this mold eight years ago with radioactive carbon dioxide ($C^{11}O_2$).[2]

The aerobic mechanism is the subject of the present work. Methods of cultivation and handling of the mold, submerged mycelium and analytical procedures are those given in detail by Foster and Davis[1, 3] and additional details will be given where necessary.

Experiments and Results.—Relation of C_2 Compounds to Fumarate Formation from Glucose: Using washed submerged mycelium the essential surface culture results of Butkewitsch and Federoff[4, 5] and Foster and Waksman[6] were confirmed, namely: aerobically ethanol accumulates in the early stages of the carbohydrate utilization, and gradually disappears, with a concomitant increase in fumarate, implying that alcohol is an intermediate between glucose and fumarate. Also confirmed was the formation of fumarate from alcohol as the sole carbon source, as well as from acetate, first noted by Takahashi and Asai in 1927.[7] A systematic study of fumarate formation from C_2 compounds (an aerobic process) was, therefore, undertaken.

Conversion of Alcohol to Fumarate: Extensive experimentation indicates the following conditions are essential for high efficiency of this conversion: (*a*) good aeration conditions, such as agitating thin layers on a reciprocal-

type shaking machine; (*b*) initial alcohol concentration of 2 to 3%; (*c*) the growth medium in which the mycelium for the alcohol experiment is obtained must be neutralized to prevent a sharp drop in pH due to fumarate formation from the sugar; if the pH falls below 5.0 the mycelium is inferior for this purpose; (*d*) phosphate cannot be used for the neutralization because it yields inferior mycelium; excess precipitated calcium carbonate has proved very satisfactory.

The washed mycelium, which is now furnished alcohol, must be allowed to become acid. In the neutral range alcohol consumption is less, and conversion to fumarate is lacking altogether or is small. Enough $CaCO_3$ can be carried over from the growth medium, trapped in the mycelial clumps, or adhering to the mucilaginous coatings on the hyphae, so as to maintain the pH high enough to suppress fumarate formation from alcohol. Washing the mycelium briefly two or three times in 0.2 *N* HCl dissolves this $CaCO_3$ without injuring the mycelium. Excessive contact with the HCl is injurious. After a water wash a portion of the mycelium placed in water and agitated violently should show no $CaCO_3$ shaken loose and settling out. The optimum acidity for conversion of alcohol to fumaric acid is about pH 3 to 4, yet maximum conversions are obtained only when the pH initially is 6 to 7 and is allowed to fall due to fumaric acid accumulation in the now unbuffered medium; under good conditions the value falls to about pH 2.5 before the conversion of alcohol is affected harmfully.

In properly conducted experiments aseptic techniques are unnecessary, due to the high acidity which develops. In these experiments, 1.2 g. wet weight (= 0.2 g. dry wt.) mycelium was used per 25 ml. of 2% ethanol in 250-ml. Erlenmeyer flasks. After 4 to 5 days shaking at 28°C. and correction for small evaporation losses, filtrates from these cultures contain from 4 to 6 mg. free fumaric acid per ml. The highest we have obtained is 7.9 mg. per ml. Doubtless higher amounts could be obtained by partial neutralization. Generally ethanol consumption amounts to 4 to 10 mg. per ml. Analytical data show this is equivalent to about 50 to 80% weight conversion of ethanol to fumaric acid. This represents approximately 40 to 60% molar conversion efficiency. Actually, the three highest values we have observed have been weight conversion = 84, 86 and 91% (= molar conversions of 67, 68 and 72%). Fumarate is never obtained from mycelium in the absence of substrate (alcohol).

Two things are remarkable about this process—the high yields of fumaric acid and the purity of the product. Paper chromatography (solvent = butanol-propionic acid)[8] of a culture filtrate containing about 500 μg. fumaric acid showed no other detectable acid spots under our conditions. This means that other metabolic acidic impurities were present in amounts less than about 1% of the amount of fumaric acid. However, after substantial concentration, namely, ether extraction and

crystallization of fumaric acid from a water solution of the residue, malic acid could be identified in the mother liquor from the crystallization. It showed an acid spot of RF = 0.43 on a paper chromatogram. The purified fumaric acid was identified as such by melting point and the $KMnO_4$ unsaturation test.

Acetate Conversion: The conversion of acetate to fumarate has been studied under a great variety of conditions, the best weight conversion never exceeding 30% (= 29% on molar basis). Neutral conditions are essential here, as utilization of acetate is greatly reduced on the acid side and fumarate formation is not observed at all. In confirmation of Butkewitsch and Federoff's[9, 10] studies with surface pads, some succinic acid is also formed with submerged mycelium. In one of our experiments 7.2 mg. acetate per ml. was consumed, yielding 1.92 mg. fumarate and 0.26 mg. succinate. The succinate was measured manometrically using a succinic dehydrogenase preparation from pig heart.

The discrepancy in yields from alcohol and acetate make it likely that acetic acid itself is not the substance undergoing conversion to fumarate, at least in the pathway from ethanol. An active C_2 compound probably is generated more efficiently from ethanol than from acetate, and though we have established that phosphate is essential for the conversion,[11] assiduous tests for acetyl phosphate[12] have been consistently negative, both on filtrates and ground mycelium extracts.

Conversion of Succinate: Fumarate formation from C_2 compounds and the fact that succinate accumulates in acetate cultures implies the intermediary formation of succinate followed by dehydrogenation to fumarate.

Demonstration of the dehydrogenation reaction is, therefore, essential for the premise that fumarate formation from C_2 compounds proceeds via succinate. Living *Rhizopus nigricans* mycelium is inert toward succinic acid, when tested manometrically for oxygen uptake. This is true at acid or neutral pH values. However, desiccated mycelium actively oxidizes succinate, indicating that permeability limitations account for the negative results with living mycelium. Judging from the amount of oxygen taken up, the oxidation of succinate does not proceed beyond fumarate in desiccated preparations, which by themselves display some endogenous O_2 consumption. In one experiment with 0.01 mM. succinate in phosphate buffer at pH 7.4, the oxygen uptake leveled off sharply to the endogenous rate, at which point the following O_2 uptake values were obtained: endogenous flask = 96 μl.; succinate flask = 200 μl.; difference due to succinate = 104 μl. O_2; theoretical for oxidation of succinate to fumarate = 112 μl. O_2. Each vessel had 30 mg. dry mold material.

Experiments on CO_2 Fixation: Because this organism can synthesize appreciable amounts of fumarate via CO_2 fixation with glucose as the substrate,[1] examination of CO_2 fixation mechanisms appeared desirable in

the alcohol experiments. A double fixation, namely, $C_2 + CO_2 \rightarrow C_3$; $C_3 + CO_2 \rightarrow C_4$ would be involved, and of exceptional efficiency. The following experiments appear to preclude these mechanisms: (*a*) fumarate formation is undiminished in a CO_2-free atmosphere secured in a closed system by the presence of alkali and partial vacuum; (*b*) fumarate formation is not accelerated or enhanced by elevated CO_2 tensions; (*c*) experiments with radioactive carbon dioxide ($C^{14}O_2$) and unlabeled ethanol show that CO_2 could not possibly account for the major portion of the total fumarate formed. Acid-permanganate degradation (see later) of the fumaric acid formed in this experiment showed that the radioactivity was predominantly in the carboxyl groups as compared to methine carbon. In view of the fact that this mold has been demonstrated to possess oxalacetic acid decarboxylase, it is almost certain that the carboxyl radioactivity results from reversible decarboxylation of oxalacetate arising from fumarate via malate. Carbon dioxide is, therefore, a negligible factor in synthesis of fumarate from alcohol.

Theoretical Consideration of the Above Results with Respect to a Tricarboxylic Acid Cycle Mechanism: For two reasons the yield data seem to eliminate the tricarboxylic acid cycle in the formation of the C_4 dicarboxylic acids (fumaric).

First, theoretical molar yields of C_4 from C_2 via a C_6-cycle are 67%. In the mold system, yields equaling and possibly exceeding these have been obtained. That the Krebs cycle could operate in this case at its theoretical efficiency in producing C_4 is improbable, due to other degradation outlets for the intermediates. The fumarate yields from alcohol are in reality substantially higher than those reported above because they are based on total alcohol which had disappeared, after corrections from suitable evaporation controls. Actually some of the alcohol was assimilated by the mold mycelium. In one alcohol experiment the dry weight increase in mold mycelium was equivalent to 15% of the alcohol which had been consumed. Since this occurred in the absence of a nitrogen source and minerals, it is considered to represent oxidative assimilation.

Second, for each molecule of C_4 formed, the tricarboxylic acid cycle would require a C_4 molecule to begin with. This would have to come from ethanol, i.e., a C_2, which is the only substrate available. Thus, a functioning Krebs cycle in this system would still require a C_4 synthesis from C_2 by some extremely efficient mechanism.

Since CO_2 fixation has been eliminated (see above) as a major mechanism, attention was, therefore, directed to a consideration of a $2C_2$-condensation origin of the fumarate. None of the above experimental data are inconsistent with this possibility, and indeed, the yield data are strongly suggestive of this mechanism. Labeled ethanol makes it possible to put this theory to the final test.

Experiments Using C^{14}-Labeled Ethanol.—Methods: Methyl-labeled ethanol and carbinol-labeled ethanol were obtained from Tracerlab, Inc. To avoid volatilization hazards, as well as to obtain CO_2 measurements, these experiments were conducted in 10-liter desiccators with ordinary air atmosphere. Other details were exactly as in the previous experiments with non-labeled ethanol.[1, 3] The cocks were opened daily to compensate for oxygen consumption. Only one flask was placed in a desiccator. The whole apparatus, with the flask fastened in position was placed on a shaker. Both kinds of labeled ethanol were run with portions of the same batch of mycelium and at the same time for 4 days. The radioactive alcohols were diluted with ordinary ethanol to a final concentration of 2%. At the end of the experiments a 300-ml. aliquot of the gas phase was removed with an evacuated bulb, the CO_2 absorbed in alkali, precipitated and weighed as $BaCO_3$ on a porcelain filter disc. Radioactivity measurements were made directly from the disc with a mica window Geiger-Müller counter. All radioactivity measurements on $BaCO_3$ were measured as close as possible to infinite thickness and corrected for self-absorption when necessary. All counts actually measured were at least 10 times the background, and most were 20 to 200 times. Radioactivity of other organic compounds was measured as $BaCO_3$ obtained from wet oxidation using persulfate-$AgNO_3$.[13] The liberated CO_2 was absorbed in NaOH and collected as $BaCO_3$ by addition of $BaCl_2$.

At time of analysis the culture with methyl-labeled ethanol had 5.4 mg. fumaric acid per ml., and that with carbinol-labeled ethanol 5.7 mg. per ml., determined by a mercurous fumarate method modified from Stotz.[14] Aliquots of the clear culture filtrates were adjusted to pH ~10 and about two-thirds the volume distilled off. The residue was acidified to pH ~2.0 with H_2SO_4 and extracted with ether overnight in a Kutscher-Steudel extractor. The ether in the extract was evaporated, and all remaining traces of volatile matter removed by maintaining the residue under high vacuum at 80°C. for 30 minutes. The light tan deposit of fumaric acid was dissolved in hot water, filtered and concentrated in an air stream on a hot plate to incipient crystallization. After standing in a refrigerator overnight, the crystals of radioactive fumaric acid were collected and washed on a sintered glass funnel. Yield from the carbinol-labeled alcohol was 52 mg. and from the methyl-labeled ethanol 32 mg. Both preparations melted at 274–275°C. in a sealed tube as compared to 279.5°C. for pure fumaric acid under our conditions.

About 5 mg. of each kind of labeled fumaric acid was combusted for total radioactivity and another 10 to 12 mg. degraded with acid-permanganate for location of the labeled carbons. Allen and Ruben[15] have shown that this oxidation liberates 3 moles CO_2 and 1 mole of formic acid per mole of fumarate, and that the formic acid is derived exclusively from

one of the methine-carbons of the fumarate. The other methine-carbon and the two carboxyls make up the three CO_2 liberated. In confirmation, we have, with unlabeled fumarate, identified formic acid as a product of this oxidation under our conditions by ether extraction and its Duclaux distillation constants. Formate separation also has been done by steam distillation direct from the oxidation mixture after destruction of the residual $KMnO_4$ with H_2O_2. The oxidation mixture contained 11 mg. fumaric acid in 6 ml., 20 ml. 0.3 *N* $KMnO_4$ and 14 ml. 3 *N* H_2SO_4. The oxidation is complete in less than 10 minutes at room temperature, but the formate slowly is further oxidized. The oxidations were carried out in 160-ml. capacity Warburg vessels connected to manometers containing mercury, and when theoretical amounts of CO_2 had been liberated (= three-fourths of the fumarate carbon) the flask was placed in ice water to retard formate oxidation. Alkali was introduced through the vent in one side arm, and the CO_2 absorbed. The oxidation mixture was removed for formate separation, and the CO_2 precipitated as $BaCO_3$ and taken for radioactivity measurements.

In the case of radioactive fumarate, inactive carrier formic and acetic acids (0.3 mE. of each) were added prior to ether extraction and Duclaux distillation. Two successive extractions with 10 volumes of ether netted about 90% recovery of the acids. The ratios of acetic/formic acid obtained in the Duclaux distillations from both kinds of labeled fumaric acid were so shifted by the ether extracts that formic acid definitely must have been contributed by the degradation of fumaric acid. The Duclaux constants are indispensable in ascertaining the radioactivity of the formic acid produced from fumarate. Radioactivity of the various Duclaux fractions should be proportional to the characteristic formic acid values in a Duclaux distillation, otherwise the radioactivity in the preparation cannot be due to formic acid. After the titration values were obtained, the various Duclaux fractions were oxidized to CO_2 for radioactivity determinations.

Results with Fumaric Acid from Carbinol-Labeled Ethanol: The formic acid in this case was devoid of radioactivity even when measured as gaseous CO_2 with the vibrating reed electrometer which is $\sim$10 times more sensitive than our solid counting procedure. There was a small amount of steam distillable radioactivity ($\sim$2% of the total) but it doubtless came from impurities in the fumarate, for it bore no relation to the formate distillation curve. The fumaric acid itself had a specific activity of 1.2 (counts/sec./mg. $BaCO_3$), located entirely in the carboxyls, in view of the inactivity of methine-carbon. *This experiment demonstrates conclusively that a direct C_2 condensation has taken place, and furthermore, that no other reactions took place whereby carbinol-carbon of ethanol is converted to methine-carbon of fumaric acid.* That is, no mixing takes place. It means the following conversion must have occurred:

$$\begin{matrix} CH_3C^{14}H_2OH \\ CH_3C^{14}H_2OH \end{matrix} \rightarrow \text{Intermediate I} \rightarrow \text{Intermediate II} \rightarrow \begin{matrix} CH{-}C^{14}OOH \\ \| \\ HOOC^{14}{-}HC \end{matrix}$$

Ethanol Fumaric Acid

Based on the fact that phosphate and succinate appear to be involved, according to the earlier experiments, the supposition is made that Intermediate I is an "active" C_2 compound, possibly phosphorylated, and Intermediate II is the primary product of the condensation reaction, presumably an "active" succinate.

The starting radioactive alcohol used in this experiment had a specific activity of 1.67. The reduced specific activity (1.2) of the fumarate formed from this alcohol could result only from a dilution of the radioactive carboxyls by inactive carboxyls. The probable mechanism: some of the alcohol is oxidized to CO_2. The CO_2 at the end of the experiment had a specific activity of 1.2, representing inactive CO_2 from methyl carbons as well as endogenous respiration, and radioactive carbon from carbinol. Thus, due to dilution, the specific activity of the CO_2 is much less than that of the carbinol group of the alcohol ($2 \times 1.67 = 3.3$). After fumarate is formed via direct C_2 condensation, the initially highly radioactive carboxyl groups are diluted with lower specific activity CO_2, probably via reversible exchange by oxalacetate decarboxylase, an enzyme known to be active in this organism.[1]

Results with Fumaric Acid from Methyl-Labeled Ethanol: The formic acid derived from this fumarate was decidedly radioactive, as was the CO_2 representing three-fourths of the fumarate. This latter contains, of course, one methine carbon, equivalent to the formic acid carbon. The radioactivity was unquestionably due to formic acid, since radioactivity measurements precisely paralleled titration values of the Duclaux fractions representing pure formic acid. The last fraction, the most accurate because of its size, contained by titration 65% of the total formic acid distilled. This fraction also was found to contain 65% of the total radioactivity of the formic acid.

The specific activity of the starting ethanol in this experiment was 1.83, and that of the isolated fumaric acid 2.18. This increase can be accounted for only by entrance of labeled carbon into the initially non-radioactive carboxyls of the fumarate. Here, also, an exchange between labeled CO_2 and the fumarate carboxyls must have occurred, again probably involving the participation of the oxalacetate decarboxylase in this organism. The labeled CO_2 had a specific activity of 0.8, and was derived from oxidation of some ethanol. The incorporation of $C^{14}O_2$ into unlabeled carboxyl naturally results in increased specific activity of the fumarate. It is interesting that the exchange reaction in the carbinol-labeled alcohol experiment

reduced the specific activity of the fumarate formed, and increased it in this experiment. These experimentally observed results are, therefore, what one would expect.

The specific activities of the methine-carbon and carboxyl-carbon of this fumarate were 3.6 and 0.7, respectively, as determined by measurements on formate and CO_2 from permanganate oxidation. Since the specific activity of the methyl group of the starting alcohol was determined to be 3.66, it is evident that a *direct conversion of the ethanol methyl group to fumarate methine group must have occurred.*

Discussion.—In recent years experiments on bacteria, yeast, fungi and animal tissue have suggested strongly the existence of the 2C condensation reaction (Thunberg-Wieland condensation). Although the evidence has, up to this point, been held in some dispute (see comprehensive literature reviews and critical analyses by Wood,[16] and Bloch,[17]) the experiments in this paper decisively establish the $2C_2$ condensation reaction. Thus, a third mechanism of formation of the C_4 dicarboxylic acids is added to the other two generally accepted as functioning in aerobic cellular respiration: CO_2 fixation yielding oxalacetic acid, and decarboxylation of α-ketoglutaric yielding succinate. Just what part, if any, the $2C_2$ condensation plays in various living systems in furnishing (eventually) oxalacetic acid for the tricarboxylic acid respiratory cycle is yet to be ascertained.

Apart from the reaction itself, of particular significance appear to be the results indicating entrance of CO_2 into carboxyl groups of the fumaric acid which itself is synthesized via a mechanism *not* concerned with CO_2 fixation. This CO_2 entrance is assumed to occur through reversible decarboxylation of pre-existing oxalacetate (or malate) in equilibrium with the fumarate. It would be of interest to determine if this mode of entrance of CO_2 could account for some of the carboxyl-labeled organic acids formed in the numerous Krebs type experiments which have heretofore been done with labeled CO_2 or $NaHCO_3$.

In view of the prominent rôle C_2 compounds play in the metabolism of fungi,[18] it is not surprising that the "$2C_2$ condensation reaction" should have a special significance in these organisms. With adequate oxygen, carbohydrate in mold cultures is oxidized via oxidative decarboxylation of pyruvate, the resulting active C_2 (acetyl phosphate?) forming organic acids and/or CO_2 eventually. However, the physical nature of mold mycelium in masses almost automatically limits oxygen availability during carbohydrate utilization, and anaerobic alcoholic fermentation ensues. Upon exhaustion of carbohydrate, the mold now slowly oxidizes the alcohol, generating organic acids and/or CO_2 just as it does from pyruvate directly. This mechanism whereby the mold eventually obtains its full quota of energy and carbon from the original carbohydrate and accumulates acids is possible because ethanol oxidation yields apparently the

same or similar active C_2 fraction as pyruvate oxidation. The oxidation merely proceeds via a roundabout route, which has been named the "alcohol excursion." These results also indicate why alcohol has so often been considered disputably the intermediate in organic acid formation from sugars by fungi.

The formation of pure fumaric acid in high yields labeled predominantly in methine-carbons should make possible the availability of similarly labeled analogs, such as oxalacetic acid, malic acid, succinic acid, aspartic acid, α-ketoglutaric acid, etc., having value as tracers in metabolism studies. It also provides now the only feasible method of synthesizing methine-labeled maleic anhydride, a vital intermediate in chemical synthesis of many suitably labeled ring-type organic compounds, including carcinogens.

Summary.—Conditions for obtaining high yields of fumaric acid from ethanol by *Rhizopus nigricans* are specified. Yields of fumaric acid were so high that the tricarboxylic acid cycle as a mechanism of formation of fumarate is excluded. Yields of fumarate from acetate are one-third those from alcohol. Experiments with unlabeled ethanol and C^{14}-labeled carbon dioxide eliminate a CO_2 fixation mechanism of fumarate synthesis. The mold possesses an enzyme which oxidizes succinate to fumarate.

Fumarate formed from C^{14} methyl-labeled ethanol contains the same specific activity in the methine-carbon as that of the methyl group from the starting ethanol. The carboxyls contain a small amount of radioactivity due to a reversible decarboxylation reaction probably involving oxalacetate decarboxylase.

Fumarate from C^{14} carbinol-labeled ethanol contained no radioactivity in the methine-carbons, but abundantly in the carboxyls. All the evidence proves unequivocally that the C_4 dicarboxylic acid synthesis occurs by direct $2C_2$ condensation as hypothesized originally by Thunberg. The implications of this reaction in the tricarboxylic acid cycle of respiration and in synthesis of tracer intermediates for biochemistry and synthetic organic chemistry are discussed.

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† On leave of absence from the University of Texas.

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GEOMETRY OF REDUCED MOMENT SPACES

BY S. KARLIN AND L. S. SHAPLEY

THE RAND CORPORATION, SANTA MONICA, CALIFORNIA

Communicated by J. von Neumann, October 7, 1949

1. A normalized distribution function on the closed unit interval $[0, 1]$ is any real, non-decreasing function $\phi(t)$, continuous to the right, with $\phi(-0) = 0$, $\phi(1) = 1$. The distribution with saltus of one at t and constant elsewhere is denoted by I_t. The nth *moment* of ϕ is

$$\mu_n(\phi) = \int_{-0}^{1} t^n d\phi(t).$$

The region of n-dimensional Euclidean space comprising the points $x_i = \mu_i(\phi)$, $i = 1, 2, \ldots, n$, for all distributions ϕ is the *reduced moment space* $\mathfrak{D}^n$. The region comprising the points of the form $x_i = \mu_i(I_t) = t^i$ is a twisted curve, denoted by $\mathfrak{C}^n$. $\mathfrak{D}^n$ is closed and convex, and for $n \geq 2$ its set of extreme points is precisely $\mathfrak{C}^n$.

THEOREM. *If x is in the boundary of $\mathfrak{D}^n$ then there is a unique distribution ϕ with moments $\mu_i(\phi) = x_i$, and x can be represented as a convex combination of extreme points in just one way, namely*

$$x_i = \int_{-0}^{1} \mu_i(I_t) d\phi(t).$$

If x is interior to $\mathfrak{D}^n$, then the associated distribution and the convex representation are not unique.

Denote by $\bar{b}(x)$ the number of extreme points participating in the convex representation of a boundary point x of $\mathfrak{D}^n$, and $b(x)$ the same but with the points $(0, 0, \ldots, 0)$ and $(1, 1, \ldots, 1)$ counted half. Let $\mathfrak{L}(x)$ be the common intersection of all the supporting planes to $\mathfrak{D}^n$ at x; let $a(x)$ denote the dimension of $\mathfrak{L}(x)$, $c(x)$ the dimension of $\mathfrak{L}(x) \cap \mathfrak{D}^n$.

THEOREM. *If x is any boundary point of $\mathfrak{D}^n$ then*

$$2b(x) = a(x) + 1,$$
$$\bar{b}(x) = c(x) + 1.$$

By a suitable extension of the definitions, these relations can be made to hold for points interior and exterior to $\mathfrak{D}^n$. In particular, there are two distinct "minimal" representations of the interior points, with $b(x) = (n + 1)/2$, corresponding to the representations of two boundary points of $\mathfrak{D}^{n+1}$. The boundary of $\mathfrak{D}^n$ may be partitioned into $2n$ disjoint "faces," $\overline{\mathfrak{A}}_a^n$ and $\underline{\mathfrak{A}}_a^n$, of surface dimension a, $a = 0, 1, \ldots, n - 1$, where $a(x) = a$ for $x \in \overline{\mathfrak{A}}_a^n$ or $\underline{\mathfrak{A}}_a^n$. $c(x)$ is constant over these faces, and indicates the order of curvature of the surface. For the entire boundary, $a - 1 \leq 2c \leq a + 1$.

2. The set of polynomials $P(t)$ of degree n or less which are non-negative over [0, 1] may be conceived as a conical region in the $(n + 1)$-dimensional Euclidean space of the coefficients. If the polynomials are normalized by

$$\int_0^1 P(t)dt = 1$$

a convex, closed, n-dimensional region $\mathfrak{P}^n$ is obtained. Its extreme points are precisely those polynomials that have n roots (counting multiplicity) in [0, 1]: they fall into two disjoint components distinguished by the sign of the leading coefficient. The entire region $\mathfrak{P}^n$ is spanned by extreme points taken two at a time; in fact:

THEOREM. *If P is in $\mathfrak{P}^n$ then there is a unique representation*

$$P(t) = \alpha \prod_{j=1}^{m} (t - t_{2j-1})^2 + \beta t(1 - t) \prod_{j=1}^{m-1} (t - t_{2j})^2, \text{ if } n = 2m,$$

$$P(t) = \alpha t \prod_{j=1}^{m} (t - t_{2j})^2 + \beta(1 - t) \prod_{j=1}^{m} (t - t_{2j-1})^2, \text{ if } n = 2m + 1,$$

with $\alpha \geq 0$, $\beta \geq 0$, $0 \leq t_1 \leq \ldots \leq t_{n-1} \leq 1$. P is interior to $\mathfrak{P}^n$ if and only if all of the inequalities are strict

Denote by $b(P)$ the number of distinct roots of $P(t)$; $\bar{b}(P)$ the same counting roots at 0 and 1 as half. Then for P on the boundary of $\mathfrak{P}^n$:

$$\bar{b}(P) = n - a(P)$$
$$2b(P) = n - c(P),$$

$a(P)$ and $c(P)$ being defined geometrically as in section 1. The boundary of $\mathfrak{P}^n$ may be partitioned into $2n$ disjoint components $\overline{\mathfrak{C}}_c^n$ and $\underline{\mathfrak{C}}_c^n$, $c = 0, 1, \ldots, n - 1$, with $c(P) = c$ for P in $\overline{\mathfrak{C}}_c^n$ or $\underline{\mathfrak{C}}_c^n$.

3. A *convex cone* in $(n + 1)$-dimensional space is a set which contains the point $\alpha x + \beta x'$ whenever it contains x and x', α and β being any non-negative real numbers.[1] The *conjugate* cone C^* to a convex cone C is the set of points y which satisfy

$$\sum_{i=0}^{n} x_i y_i \geq 0 \qquad \text{all } x \in C.$$

C^* is convex closed, and if C is closed then $C^{**} = C$.

If $\mathfrak{D}^n$ and $\mathfrak{P}^n$ are made into convex cones in $(n + 1)$-dimensional space by dropping the two normalizing conditions $\int d\phi = 1$, and $\int P dt = 1$, then they are mutually conjugate. Geometrically stated, this means that the coördinates of a boundary point of $\mathfrak{D}^n$ are the coefficients of a plane of support to $\mathfrak{P}^n$, and vice versa. Interior points correspond to separating

planes, exterior points to secant planes. If x is in the boundary of $\mathfrak{D}^n$ and if P is interior to the (convex) set of points in the boundary of $\mathfrak{P}^n$ which generate planes of support to $\mathfrak{D}^n$ at x, then P is said to be *conjugate* to x.

THEOREM. *If x is conjugate to P, or P conjugate to x, then*

$$a(x) + c(P) = c(x) + a(P) = n - 1,$$
$$b(x) = b(P), \qquad \bar{b}(x) = \bar{b}(P).$$

The duality of conjugate cones is fundamental in the geometrical interpretation of the theory of two-person, zero-sum games. Motivation for the present study came from the consideration of games described by polynomial payoff functions over the unit square.

4. In this section, results will be given only for the case $n = 2m$. Analogous results hold for odd dimensions.

The duality just described leads easily to the well-known fact,[2] that $\mu_0 = 1, \mu_1, \ldots, \mu_{2m}$ are the moments of a distribution if and only if the quadratic forms

$$\sum_{i,j=0}^{m} x_i x_j \mu_{i+j} \text{ and } \sum_{i,j=1}^{m} x_i x_j (\mu_{i+j-1} - \mu_{i+j})$$

are non-negative. The first principal minors are

$$\underline{\Delta}_k = \begin{vmatrix} \mu_0 & \cdots & \mu_k \\ \cdot & & \\ \cdot & & \\ \cdot & & \\ \mu_k & \cdots & \mu_{2k} \end{vmatrix} \text{ and } \bar{\Delta}_l = \begin{vmatrix} \mu_1 - \mu_2 & \cdots & \mu_l - \mu_{l+1} \\ \cdot & & \\ \cdot & & \\ \cdot & & \\ \mu_l - \mu_{l+1} & \cdots & \mu_{2l-1} - \mu_{2l} \end{vmatrix}$$

$k = 0, 1, \ldots, m$, $l = 1, 2, \ldots, m$, respectively. These determinants may be used to characterize the faces $\bar{\mathfrak{A}}_a^{2m}$, $\underline{\mathfrak{A}}_a^{2m}$ of $\mathfrak{D}^{2m}$. In fact, if x is in $\bar{\mathfrak{A}}_a^{2m}$ then, setting $b = (a + 1)/2$,

$$\underline{\Delta}_k > 0, \quad 1 \le k \le b; \qquad \underline{\Delta}_k = 0, \quad b < k \le m;$$
$$\bar{\Delta}_l > 0, \quad 1 \le l < b; \qquad \bar{\Delta}_l = 0, \quad b \le l \le m.$$

If x is in $\underline{\mathfrak{A}}_a^{2m}$ then interchange upper and lower bars.

If an interior point $(\mu_1, \ldots, \mu_{2m-1})$ of $\mathfrak{D}^{2m-1}$ is given, then the extreme values possible for μ_{2m} are found by solving $\underline{\Delta}_{2m} = 0$ and $\bar{\Delta}_{2m} = 0$. The lower value, designated $\underline{\mu}_{2m}$ comes from the first equation, the upper, $\bar{\mu}_{2m}$, from the second. The supporting planes at the opposite boundary points $(\mu_0, \ldots, \underline{\mu}_{2m})$ and $(\mu_0, \ldots, \bar{\mu}_{2m})$ are unique and correspond to the polynomials $[P_{\underline{\Delta}_m}(t)]^2$ and $t(1 - t)[P_{\Delta^m}(t)]^2$ of $\mathfrak{P}^{2m}$ (when normalized), where

$$P_{\Delta_m}(t) = \begin{vmatrix} \mu_0 & \cdots & \mu_{m-1} & 1 \\ \mu_1 & & & t \\ \cdot & & & \cdot \\ \cdot & & & \cdot \\ \cdot & & & \cdot \\ \mu_m & \cdots & \mu_{2m-1} & t^m \end{vmatrix},$$

$$P_{\Delta_m}(t) = \begin{vmatrix} \mu_1 - \mu_2 & \cdots & \mu_{m-1} - \mu_m & 1 \\ \mu_2 - \mu_3 & & & t \\ \cdot & & & \cdot \\ \cdot & & & \cdot \\ \cdot & & & \cdot \\ \mu_m - \mu_{m+1} & \cdots & \mu_{2m-2} - \mu_{2m-1} & t^{m-1} \end{vmatrix}.$$

The spectra of the unique distribution $\underline{\psi}$ and $\bar{\psi}$ corresponding to these opposite boundary points are the roots of $P_{\Delta_m}(t)$ and $t(1-t)P_{\Delta^m}(t)$, respectively. The two sets of roots interlock strictly (cf. the representation of section 2) and the cumulative weights of the two distributions interlock as well, so that $\underline{\psi}(t) - \bar{\psi}(t)$ has a sign change at each saltus point of $\underline{\psi}$ and $\bar{\psi}$.

The polynomials P_{Δ_k}, $k = 0, 1, \ldots, m$, are orthogonal with respect to the weight factor $d\underline{\psi}(t)$, while P_{Δ^l}, $l = 1, 2, \ldots, m$, are orthogonal with respect to $t(1-t)d\bar{\psi}(t)$. These systems of polynomials, together with the analogs for odd n, are the basis for a new geometric approach to the theory of orthogonal polynomials.

THEOREM. *There is a 1:1:1 correspondence between (a) the interior of* $\mathfrak{D}^{2m-1}$, *(b) the set of ordered pairs of polynomials* $(P_{\Delta_m}, P_{\Delta^m})$, *and (c) the open simplex of strictly interlocking roots* $0 = t_0 < t_1 < \ldots < t_{2m} = 1$.

The representation of section 2 is thus linked to the polynomials of this section and to the interior of $\mathfrak{D}^{n-1}$.

5. In this section certain geometric features of the reduced moment space $\mathfrak{D}^n$ and its dual $\mathfrak{P}^n$ will be given. $\mathfrak{D}^n$ can be inscribed in a very natural simplex $\mathfrak{S}^n$ whose vertices $x^{(k)}$ are given by

$$x_i^{(k)} = \frac{\binom{k}{i}}{\binom{n}{i}} \qquad i = 1, 2, \ldots, n;\ k = 0, 1, \ldots, n.$$

Its dual is the simplex inscribed in $\mathfrak{P}^n$ spanned by the polynomials

$$\lambda_{n,k}(t) = \binom{n}{k} t^k (1-t)^{n-k} \qquad k = 0, 1, \ldots, n,$$

(which must be normalized by the factor $n + 1$). The barycentric coordinates with respect to $\mathfrak{S}^n$ of a point x of $\mathfrak{D}^n$ are

$$\lambda_{n,k} = \int_0^1 \lambda_{n,k}(t)d\phi(t) \qquad k = 0, 1, \ldots, n,$$

where ϕ is a distribution having moments $\mu_i(\phi) = x_i$, $i = 1, 2, \ldots, n$. This gives a geometric interpretation to the quantities $\lambda_{n,k}$ which play so important a rôle in the literature on moment theory.[3] The relation of $\mathfrak{S}^n$ to $\mathfrak{D}^n$ leads to several qualitative results on moment spaces, and in particular gives a simple geometrical proof of Hausdorff's Theorem.

An interesting point in the interior of each $\mathfrak{D}^n$ is that given by the continuous distribution $dt/\sqrt{t(1-t)}$ whose moments

$$\mu_i = \frac{1 \cdot 3 \cdot \ldots \cdot 2n-1}{2 \cdot 4 \cdot \ldots \cdot 2n}$$

satisfy, for any n, $\mu_n = (\underline{\mu}_n + \bar{\mu}_n)/2$. The supporting planes at $(\mu_1, \mu_2, \ldots, \underline{\mu}_n)$ and $(\mu_1, \mu_2, \ldots, \bar{\mu}_n)$ are parallel, another unique property of this moment sequence. The polynomials $P_{\underline{\Delta}m}$, $P_{\bar{\Delta}m}$, $n = 2m$, for these moments are (up to a constant factor) the Tchebycheff polynomials of the first and second kind.

The center of gravity of the simplex $\mathfrak{S}^n$ is given by the rectangular distribution $\phi(t) = t$, whose moments $1, {}^1/_2, {}^1/_3, \ldots$ give rise in similar fashion to the Legendre and the first associated Legendre polynomials. The centroid of the dual simplex is $P(t) \equiv 1$.

Finally, the n-dimensional volume of $\mathfrak{D}^n$ has the interesting value

$$V_n = \prod_{i=1}^{n} B(i, i),$$

where B is the beta function.

6. A similar account can be made for moment spaces based on an arbitrary finite set of moments $\{\mu_{i_\nu}\}$. With certain adaptations, most of the foregoing analysis and geometry can be carried over to infinite moment sequences, and to distributions over the infinite intervals $[0, \infty]$ and $[-\infty, \infty]$. These results and their proof, and additional theorems unstated here, will be published elsewhere.

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ON n-DIMENSIONAL CONCEPTS OF BOUNDED VARIATION, ABSOLUTE CONTINUITY AND GENERALIZED JACOBIAN

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Background.—A real-valued function $x = f(u)$ may be thought of as defining a mapping $f: U^1 \to X^1$, where U^1, X^1 designate Euclidean 1-spaces (real number-lines). In this interpretation the fundamental concepts of bounded variation, absolute continuity and derivative acquire important and suggestive geometrical meaning. To obtain fruitful n-dimensional generalizations of these fundamental concepts, it is then natural to study mappings $f: U^n \to X^n$, where U^n, X^n are Euclidean n-spaces with points $u = (u_1, \ldots, u_n)$, $x = (x_1, \ldots, x_n)$. The general objective is to develop concepts of bounded variation, absolute continuity and generalized Jacobian, which yield a theory comparable in utility and scope with the classical one-dimensional theory. Various approaches to this general objective may be found in the literature. The present note is concerned with the line of thought presented in reference 1. The theory in reference 1 is developed for the case $n = 2$. For this case, the theory may be considered as essentially complete, due to the sustained efforts of many mathematicians. The generalization to the n-dimensional case represents a long-range program of great interest and great difficulty. The purpose of this note is to report on progress achieved by the writers in this general direction. We shall present in detail the precise definitions of the n-dimensional concepts of bounded variation, absolute continuity and generalized Jacobian which we use in our work, and we shall describe applications to the transformation of multiple integrals, a topic which is discussed in Part IV of reference 1 for the case $n = 2$.

Basic Concepts.—Let D_0 be a bounded domain (connected open set) in the space U^n, and let $f: D_0 \to X^n$ be a bounded continuous mapping from D_0 into the space X^n. In analogy with the 2-dimensional case it is expedient to assign to every point x of X^n an essential multiplicity relative to the mapping f. For the 2-dimensional case, various equivalent definitions of the essential multiplicity are available—in fact, four such essential multiplicities were actually used in the literature. In reference 2 these essential multiplicities are denoted by ψ, ψ^*, Ψ, Ψ^*, respectively. Since they differ from each other only on countable sets, they can be used interchangeably throughout the 2-dimensional theory. However, the corresponding essential multiplicities in n dimensions may differ from each other on sets of positive measure. Recent results of Federer[3] indicate that the multiplicity corresponding to the largest one of the four multi-

plicities just mentioned, namely Ψ^*, is the appropriate one for the n-dimensional case. In the work of Federer, the definition of this multiplicity is stated, in a very convenient manner, in terms of relative Cech cohomology groups. This essential multiplicity is used in the present note, and is denoted by K. For the concept of absolute continuity several equivalent definitions are available in the 2-dimensional case. For the n-dimensional case the choice is again restricted; we found it appropriate to adopt the definition proposed in reference 4. Finally, for the n-dimensional generalized Jacobian we found it convenient to adopt the form of the definition suggested in reference 5 for the 2-dimensional case. Precise definitions of these basic concepts follow.

The Index $\mu(x, f, D)$.—Given a bounded continuous mapping T: $D_0 \to X^n$ as above, let x_0 be any point in X^n. A domain D is termed admissible for x_0, f, D_0 if the closure cD of D is contained in D_0 and x_0 is not in the image of the frontier frD of D under f. If D is admissible for x_0, f, D_0, there exist in X^n pairs of compact sets A, B satisfying the following conditions: (i) frA is a subset of B, and B is a subset of A; (ii) $A - B$ is not empty and is connected; (iii) the image of cD under f is contained in A; (iv) the image of frD under f is contained in B; (v) x_0 is in $A - B$. Under these conditions the pair A, B is termed admissible for x_0, f, D. Assume that A, B is admissible for x_0, f, D, and consider the mapping $f|cD$: $(cD, frD) \to (A, B)$. This mapping induces a homomorphism h: $H^n(A, B) \to H^n(cD, frD)$, where H^n denotes the n-dimensional Cech cohomology group with integral coefficients. Both of the groups involved are infinite cyclic, and by a suitable orientation process one may select once for all standard generators g_D, g_{AB} for the groups $H^n(cD, frD)$, $H^n(A, B)$, respectively. Then $h(g_{AB}) = k \cdot g_D$, where k is an integer (positive, negative or zero). This integer k is defined to be the index $\mu(x_0, f, D)$; it can be easily shown to depend solely upon x_0, f, D as the notation suggests. If $\mu(x_0, f, D)$ is not zero, then D is termed an indicator domain for x_0, f, D_0. An indicator domain D is termed positive (negative) if the sign of $\mu(x_0, f, D)$ is positive (negative).

The Essential Multiplicity Function $K(x, f, D_0)$.—Let σ be a generic notation for a finite system of pairwise disjoint indicator domains D for x_0, f, D_0. Then $K(x_0, f, D_0)$ is defined to be the least upper bound of $\sum |\mu(x_0, f, D)|$ for D in σ, with respect to all systems σ. If each D occurring in the system σ is restricted to be a positive (negative) indicator domain, one obtains the definition of the multiplicity functions $K^+(x_0, f, D_0)$, $(K^-(x_0, f, D_0))$.

The Essential Maximal Model Continuum.—A continuum c is termed an essential maximal model continuum for x_0, f, D_0 if c is a component of the inverse of x_0 under f in D_0, and every neighborhood of c contains in its interior an indicator domain D which contains c. The essential set

$E(f, D_0)$ consists of all points u_0 in D_0 which belong to an essential maximal model continuum for $f(u_0)$, f, D_0.

Essential Bounded Variation, Essential Absolute Continuity.—The mapping f is said to be eBV in D_0 (essentially of bounded variation in D_0) if $K(x, f, D_0)$ is summable. The mapping f is said to be eAC in D_0 (essentially absolutely continuous in D_0) if (i) it is eBV in D_0, and (ii) for every subset b of $E(f, D_0)$ which has measure zero, it is true that the measure of the image of b under f is also zero.

The Signed Essential Multiplicity Function $\mu_e(x, f, D_0)$.—This is defined to be $K^+(x, f, D_0) - K^-(x, f, D_0)$ unless both K^+ and K^- are infinite. When f is eBV in D_0, $\mu_e(x, f, D_0)$ is defined almost everywhere in X^n and is summable. If D is an admissible domain for x_0, f, D_0, and $K(x_0, f, D)$ is finite, then the index $\mu(x_0, f, D)$ and the signed essential multiplicity $\mu_e(x_0, f, D)$ have the same value.

The Essential Generalized Jacobian $J_e(u, T)$.—Assume that f is eBV in D_0. For each open interval I in D_0, the signed essential multiplicity function $\mu_e(x, f, I)$ is defined almost everywhere in X^n and is summable. The Lebesgue integral of $\mu_e(x, f, I)$ yields a function of intervals I in D_0 which possesses a derivative almost everywhere in D_0; this derivative is the essential generalized Jacobian $J_e(u, f)$.

Application to the Transformation of n-tuple Integrals.—In terms of the preceding concepts, transformation formulae for n-tuple integrals are established which represent complete generalizations of the 2-dimensional formulae in reference 1, IV.4.4, IV.4.6, IV.4.7, IV.4.9, with the signed essential multiplicity μ_e replacing the function ν in these formulae. As a generalization of reference 1, IV.4.8, it is shown that if f is eBV in D_0, then the integral over D_0 of $|J_e(u, f)|$ is less than or equal to the integral over X^n of $K(x, f, D_0)$, the sign of equality holding if and only if f is eAC in D_0. The range of applicability of these n-dimensional transformation formulae depends upon the scope of the eAG mappings. It is shown that this class is closed under various limit processes, the results representing complete generalizations of the closure theorems in reference 1, IV.4.11, IV.4.12, IV.4.15, IV.4.16, IV.4.17, IV.4.18, IV.4.19. It is demonstrated that if f is eBV in D_0 and if the coordinate functions defining f possess complete differentials almost everywhere in D_0, then $J_e(u, f)$ agrees with the ordinary Jacobian almost everywhere in D_0. Consequently the n-dimensional transformation formulae contain, as very special cases, various special results established in previous literature. Many further results presented in Part IV of reference 1 admit of a complete generalization to n dimensions.

A detailed presentation of our work is now being prepared for publication.

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QUADRATIC RELATIONS INVOLVING THE NUMBERS OF SOLUTIONS OF CERTAIN TYPES OF EQUATIONS IN A FINITE FIELD

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In two previous papers[1] the writer considered the problem of finding the number of sets s, t, such that

$$g^{i+m_1 s} + g^{j+m_2 t} + 1 = 0, \tag{1}$$

where m_1 and m_2 are integers $0 < m_1 < p^n - 1$; $0 < m_2 < p^n - 1$; $p^n - 1 \equiv 0 \pmod{m_1}$, $p^n - 1 \equiv 0 \pmod{m_2}$, g is a primitive root in a finite field $F(p^n)$, p an odd prime, i a given integer in the set $0, 1, \ldots, m_1 - 1$; j a given integer in the set $0, 1, \ldots, m_2 - 1$, s in the set $0, 1, \ldots, m_1' - 1$; t in the set $0, 1, \ldots, m_2' - 1$; $p^n - 1 = m_1 m_1' = m_2 m_2'$. Denoting the number of possible sets s, t by $(i, j)_{m_1 m_2}$, an explicit expression in terms of p and n for the expression

$$\sum_{\substack{i=0,\ldots m_1-1 \\ j=0,\ldots m_2-1}} (i, j)_{m_1 m_2} (i + h, j + k)_{m_1 m_2},$$

in the two Cases I. $m_1 = m_2$, II. $m_1 m_2 = p^n - 1$, $(m_1, m_2) = 1$, was set up. In the present paper we obtain, using much simpler methods, similar results for m_1 and m_2 any divisors of $p^n - 1$, also quadratic relations of a different type when $m_1 = m_2$. In Hua and Vandiver[2] an expression for the number of solutions of (1) was derived, noting that the equation

$$c_1 x_1^{m_1} + c_2 x_2^{m_2} + c_3 = 0 \tag{2}$$

in $F(p^n)$ may be put in the form (1), when $c_1 c_2 c_3 x_1 x_2 \neq 0$, but the use of this form[2] is inconvenient for our present purpose. We have recourse to some results in another paper by the writer.[3]

If we keep i and j fixed the number of solutions of (1) is $m_1 m_2 (i, j)$ where (i, j) is the number of different sets s, t which are possible in the relation

(3) and in the respective ranges just mentioned for s and t. Let now $(k, l)_m$ be the number of different solutions s_1, t_1 of

$$1 + g^{k+ms_1} + g^{l+mt_1} = 0, \tag{3}$$

with m the L. C. M. of m_1 and m_2, with both s_1 and t_1 in the range 0, 1, ... $((p^n - 1)/m) - 1$. Consider the expression

$$\theta(\alpha) = \sum_{k,\,l}^{0 \text{ to } m-1} (k, l)_m \alpha^M, \tag{4}$$

where $\alpha = e^{2i\pi/m}$, and $M = u_1km/m_1 + u_2lm/m_2$. This may also be written as

$$\sum_{k,\,l}^{0 \text{ to } m-1} (k, l)_m \alpha_1^{u_1k} \alpha_2^{u_2l},$$

where $\alpha_1 = e^{2i\pi/m_1}$, $\alpha_2 = e^{2i\pi/m_2}$. Further this may be reduced to[3]

$$\psi(\alpha_1^{u_1}, \alpha_2^{u_2}) = \sum_{i,\,j} (i, j)\alpha_1^{u_1i}\alpha_2^{u_2j}, \tag{5}$$

where i ranges independently over 0, 1, ..., $m_1 - 1$ and j independently over 0, 1, ..., $m_2 - 1$, with $(k, l) = (k, l)_{m_1m_2}$ and

$$\psi(\alpha_1^{u_1}, \alpha_2^{u_2})\psi(\alpha_1^{-u_1}, \alpha_2^{-u_2}) = p^n, \tag{6}$$

with $\alpha_1^{u_1} \neq 1$, $\alpha_2^{u_2} \neq 1$, $\alpha_1^{u_1}\alpha_2^{u_2} \neq 1$, since it is known that $\theta(\alpha)\theta(\alpha^{-1}) = p^n$ under the conditions, $u_1 \dfrac{m}{m_1} \not\equiv 0 \pmod m$; $u_2 \dfrac{m}{m_2} \not\equiv 0 \pmod m$; and $u_1 \dfrac{m}{m_1} + u_2 \dfrac{m}{m_2} \not\equiv 0 \pmod m$, which reduce to the former conditions. Now for a fixed i and j, with i' and u_1 ranging independently over 0, 1, ..., $m_1 - 1$, and j' and u_2 ranging independently over 0, 1, ..., $m_2 - 1$, we have

$$m_1m_2(i, j) = \sum_{i',\,j',\,u_1,\,u_2} (i', j')\alpha_1^{u_1(i'-i)}\alpha^{u_2(j'-j)}, \tag{7}$$

for when we carry out the summation on the right with respect to the u's every term vanishes except when $i' = i \pmod{m_1}$ and $j' = j \pmod{m_2}$ simultaneously, since $\sum_{u_1} \alpha_1^{u_1k} = 0$, $k \not\equiv 0 \pmod{m_1}$ and it equals m_1 for $k = 0$, with a similar result involving α_2. Using (4), consider the relation

$$\sum_{i,\,j} m_1^2m_2^2(i, j)(i + h, j + k) = \sum_{u_1,\,u_2,\,u_1',\,u_2'}^{i,\,j} \psi(\alpha_1^{u_1}, \alpha_2^{u_2})\psi(\alpha_1^{u_1'}\alpha_2^{u_2'}) \cdot \alpha_1^{-iu_1 - iu_1' - hu_1'}\alpha_2^{-ju_2 - ju_2' - ku_2'}. \tag{7a}$$

Carrying out the summation on the right we find that every term vanishes except when $u_1 + u_1' \equiv 0 \pmod{m_1}$ and $u_2 + u_2' \equiv 0 \pmod{m_2}$ simul-

taneously, hence the right-hand member reduces to m_1m_2 times the expression

$$\sum_{u_1,\,u_2}\psi(\alpha_1^{u_1},\,\alpha_2^{u_2})\psi(\alpha_1^{-u_1},\,\alpha_2^{-u_2})\alpha_1^{hu_1}\alpha_2^{ku_2}. \tag{8}$$

Now as in the proof of Theorem I of N, we consider the five cases:

I. $\alpha_1^{u_1} = 1,\ \alpha_2^{u_2} = 1.$
II. $\alpha_1^{u_1} = 1,\ \alpha_2^{u_2} \neq 1.$
III. $\alpha_1^{u_1} \neq 1,\ \alpha_2^{u_2} = 1.$
IV. $\alpha_1^{u_1}\alpha_2^{u_2} = 1,\ \alpha_1^{u_1} \neq 1,\ \alpha_2^{u_2} \neq 1.$
V. $\alpha_1^{u_1}\alpha_2^{u_2} \neq 1,\ \alpha_1^{u_1} \neq 1,\ \alpha_2^{u_2} \neq 1.$

For Case I we find that (8) reduces to $(p^n - 2)^2$. For Case II, we find since $\psi(1,\ \alpha_2^{u_2}) = -1$ for $\alpha_2^{u_2} \neq 1$, the value $\sum_{u_2}\alpha_2^{ku_2}$ and similarly for Case III. In Case IV, using the relation $\alpha_1^{u_1}\alpha_2^{u_2} = 1$ as in the proof of (15) of N, we find that the corresponding terms reduce to $\sum_{v=1}^{d-1}\alpha_1^{(h-k)2v}$, where d is the G. C. D. of m_1 and m_2 and $m_1 = s_1d$.

For Case V we find that the corresponding terms become

$$p^n\Big(\sum_{\substack{u_1 \neq 0\\ u_2 \neq 0}}\alpha_1^{hu_1}\alpha_2^{ku_2} - \sum_{v=1}^{d-1}\alpha_1^{(h-k)s_1v}\Big).$$

Hence summing the terms for Cases I to V, inclusive, gives from

$$m_1m_2\sum_{i,j}(i,j)(i+h,j+k) = (p^n-2)^2 + \sum_{u_2\neq 0}\alpha_2^{ku_2} + \sum_{u_1\neq 0}\alpha_1^{hu_1} + \sum_{v=1}^{d-1}\alpha_1^{(h-k)vs_1} + p^n\Big(\sum_{\substack{u_1\neq 0\\ u_2\neq 0}}\alpha_1^{hu_1}\alpha_2^{ku_2} - \sum_{v=1}^{d-1}\alpha_1^{(h-k)vs}\Big). \tag{9}$$

This relation may be expressed, in terms of rational integers only, as follows:

Set

$$m_1m_2(i,j) = V \text{ and } V(i,j) = S_1$$
$$V(i,j+k) = S_2 \text{ if } \alpha_1^{ks_1} \neq 1,$$
$$V(i,j+k) = S_3 \text{ if } \alpha_1^{ks_1} = 1,$$
$$V(i+h,j) = S_4 \text{ if } \alpha_1^{hs_1} \neq 1,$$
$$V(i+h,j) = S_5 \text{ if } \alpha_1^{hs_1} = 1,$$
$$V(i+h,j+k) = S_6 \text{ if } \alpha_1^{s_1(h-k)} \neq 1,$$
$$V(i+h,j+k) = S_7 \text{ if } \alpha_1^{s_1(h-k)} = 1,$$

where in each of the above $h \not\equiv 0 \pmod{m_1}$, $k \not\equiv 0 \pmod{m_2}$. We find by the use of (9),

$$S_1 = (p^n - 2)^2 + m_1 + m_2 + d - 3 + p^n((m_1 - 1)(m_2 - 1) - d + 1)$$
$$= (p^n - 1)^2 - (p^n - 1)(m_1 + m_2 + d) + p^n m_1 m_2, \tag{10}$$

and similarly

$$S_2 = (p^n - 1)^2 - (p^n - 1)m_1, \tag{11}$$

$$S_3 = (p^n - 1)^2 - (p^n - 1)(m_1 + d), \tag{12}$$

$$S_4 = (p^n - 1)^2 - (p^n - 1)m_2, \tag{13}$$

$$S_5 = (p^n - 1)^2 - (p^n - 1)(m_2 + d), \tag{14}$$

$$S_6 = (p^n - 1)^2, \tag{15}$$

$$S_7 = (p^n - 1)^2 - d(p^n - 1). \tag{16}$$

Hence we have

THEOREM I. *Let (i, j) represent the number of sets s, t satisfying (3) with i and j fixed. s in the range $0, 1, \ldots, m_1' - 1$, t in the range $0, 1, \ldots, m_2' - 1$, g being a primitive root of the finite field $F(p^n)$, p prime, then the relations (10) to (16), inclusive, hold with m_1 and m_2 divisors of $p^n - 1$, d the G. C. D. of m_1 and m_2, with $m_1 = s_1 d$.*

In addition to expressing S_1 as the sum of squares in (10) we may obtain other expressions from the above theorem involving sums of squares. For example we have

$$\sum_{i,\,j}((i, j) - (i + h, j + k))^2 = 2S_1 - 2S_6 \tag{17}$$

where $\alpha_1^{s_1(h - k)} \neq 1$.

We now consider some other types of quadratic relations between numbers of solutions of (1) with the particular case when $m_1 = m_2 = m$. Let $\alpha = e^{2i\pi/m}$ and set

$$a_{s,\,r} = \sum_{\rho\,=\,0}^{m\,-\,1} (\rho, r + s\rho).$$

We shall show how to find an explicit expression for

$$\sum_{r\,=\,0}^{m\,-\,1} a_{s,\,r} a_{s,\,r\,+\,k} = A.$$

To effect this we employ the relation (6) and (7a) for $m_1 = m_2 = m$. We have

$$A = \sum_{\substack{\rho',\ \mu_1,\ \mu_2 \\ \mu_1',\ \mu_2'}} \psi(\alpha^{\mu_1}, \alpha^{\mu_2})\psi(\alpha^{\mu_1'}, \alpha^{\mu_2'}) \cdot \alpha^{-\rho\mu_1 - (r + s\rho)\mu_2 - \rho'\mu_1' - (r + s\rho' + k)\mu_2'} \tag{18}$$

where all the quantities involved in the summation on the right range, independently over 0, 1, . . . ,$m - 1$. The second factor in the right-hand member of this can be written in the form

$$A = \sum_{\substack{\mu_1\mu_2 \\ \mu_1'\mu_2'}} \psi(\alpha^{\mu_1}, \alpha^{\mu_2})\psi(\alpha^{\mu_1'}, \alpha^{\mu_2'}) \cdot \sum_{\rho} \alpha^{-\rho(\mu_1 + s\mu_2)} \sum_{\rho'} \alpha^{\rho'(\mu_1' + s\mu_2')} \sum_{r} \alpha^{r(\mu_2 + \mu_2')} \tag{19}$$

which reduces to zero unless

$$\begin{aligned} \mu_1 + s\mu_2 &\equiv 0 \\ \mu_1' + s\mu_2' &\equiv 0 \\ \mu_2 + \mu_2' &\equiv 0 \end{aligned}$$

modulo m, simultaneously. Hence

$$A = \sum_{\mu_2} m^3 \psi(\alpha^{-s\mu_2}\alpha^{\mu_2})\psi(\alpha^{s\mu_2}, \alpha^{-\mu_2})\alpha^{k\mu_2}$$

which in view of (6), (7), and (7a), and the known values of each ψ when $\alpha^{\mu_2} = 1$, we obtain (A. Hurwitz[4])

$$\sum_{r=0}^{m-1} a_{sr}a_{s,\,r+k} = (p^n - 4)m'; \tag{20}$$

$$\sum_{r=0}^{m-1} a_{sr}^2 = (p^n - 4)m' + p^n, \tag{21}$$

with s and $s - 1$ each prime to m, $k \not\equiv 0 \pmod{m}$, $mm' = p^n - 1$.

From (20) and (21) we obtain

$$\sum_{r=0}^{m-1} (a_{s,\,r} - a_{s,\,r+k})^2 = 2p^n \tag{22}$$

which gives m^2 representations (possibly not all different) of $2p^n$ as the sum of m squares. Similarly (17) gives analogous representations of various types of integers.

It is easy to see that we may generalize (20) to include the cases when s or $s - 1$ may not be prime to m.

[1] These PROCEEDINGS, **33,** 236–242 (1947); **34,** 62–66 (1948).

[2] *Ibid.*, **35,** 481–487 (1949). However, that paper (which will be referred to as *N*) employs a classification of various equations of the type (1) which we shall use here.

[3] These PROCEEDINGS, **32,** 317–319 (1946). Davenport and Hasse, *Jour. für die Mathematik (Crelle)*, **172,** 174 (1935) used an analogous expression involving characters and the number of solutions of (2) without the restriction $n_1n_2 \neq 0$.

[4] *Jour. für die Mathematik (Crelle)*, **136,** 390 (1909) for $n = 1$ and m an odd prime. The success of Hurwitz' method appears to depend on the fact that he restricts m in this way. His argument is quite long.

ON THE USE OF INDETERMINATES IN THE THEORY OF EXPONENTIAL SUMS

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Consider the sum

$$\psi(\alpha) = \sum_a \alpha^{\mu_1 \operatorname{ind} a + \mu_2 \operatorname{ind} (1-a)}, \tag{1}$$

where a ranges over all the elements of a finite field $F(p^n)$, of order p^n with p an odd prime, μ_1, μ_2 and $\mu_1 + \mu_2$ are integers, none divisible by m, $\alpha = e^{2i\pi/m}$, ind a is given by $g^{\operatorname{ind} a} = a$, where g is a primitive root of $F(p^n)$, $\alpha^{\operatorname{ind}(0)} = 0$, $p^n - 1 \equiv 0 \pmod m$. Many relations[1] have been given involving equation (1) but few of these express all that may be inferred from the proofs given for them. But to have given the whole story would have led to quite complicated formulas in many cases, so to illustrate the points we have in mind we start our discussion with some of the simplest relations along these lines.

It is known that

$$\psi(\alpha)\psi(\alpha^{-1}) = p^n, \tag{2}$$

if each of μ_1, μ_2, $\mu_1 + \mu_2 \not\equiv \pmod m$. If we seek the equivalent of this using indeterminates, we note that we can write

$$\psi(x)\psi(x^{m-1}) = p^n + V(x)F(x), \tag{3}$$

where $F(x)$ is the irreducible polynomial of degree $\phi(m)$, the indicator of m, with rational integral coefficients which vanishes for all the primitive roots of $x^m = 1$, and $V(x)$ is a polynomial with rational integral coefficients. The relation (3) holds since

$$\psi(x)\psi(x^{m-1}) - p^n \tag{3a}$$

vanishes for any root of $F(x) = 0$ and therefore is a polynomial which is a multiple of $F(x)$. Now for m composite $F(x)$ can be quite a complicated polynomial. *But it is possible*[2] *to prove* (2) *so that the only property of* α *employed is* $\alpha^{m-1} + \alpha^{m-2} + \ldots + 1 = 0$. Hence we are entitled to state in place of equation (3),

$$\psi(x)\psi(x^{m-1}) = p^n + W(x)\frac{x^m - 1}{x - 1}, \tag{4}$$

since equation (3a) is a polynomial which vanishes for all the roots, $\neq 1$, of $x^{m-1} + x^{m-2} + \ldots + 1 = 0$. Now we first give a very simple example

to show that (4) is more powerful than (3). If we substitute $x = 1$ in equation (3), we obtain, if we let $m = q^r$, $r > 1$, q prime

$$\psi(1)^2 = p^n + qW(1) \equiv p^n \pmod{q},$$

whereas if we substitute $x = 1$ in equation (4), we have $x(1)^2 \equiv p^n \pmod{q^r}$.

Among the things we can do with such identities as equations (3) and (4) is to differentiate them. Starting with equation (3), we have for $m = q^2$, differentiating with respect to x, calling $A(x)$ the ordinary derivative of each member and setting $x = 1$ in the result,

$$A(1) \equiv qV'(1) \pmod{q^2}.$$

The same procedure with equation (4) gives

$$A(1) \equiv 0 \pmod{q^2}.$$

Hence equation (4) has again given us more information than (3).

The above remarks show that when m is prime the relation (3) is the same as (4) so that the use of an indeterminate yields nothing more than we obtain from equation (3).

The form (1) is an expression associated with cyclotomy but the writer has noted that many other types of exponential sums, apparently not related to this topic, have been considered by various writers and results derived concerning them *with the use of the single property*

$$1 + \alpha + \ldots + \alpha^{m-1} = 0,$$

if mth *roots of unity are employed.* Hence our observations will apply to these latter types also. On the other hand, such questions as the factorization into ideals of $(\psi(\alpha))$ makes use of the fact that the degree of the field is $\phi(m)$, the indicator of m, so that we may expect nothing new, by the use of our methods, along this line.

Before proceeding to the proof of some more general results we shall explain the idea of formal exponential differentiation[3] which we wish to employ. We first consider a polynomial $f(x)$ in x with coefficients in R, where R is the ring of integers in $K(\gamma, \alpha)$ an algebraic field including α, or considering x as an integral transcendental adjunction, we consider a quantity in $R[x]$. We first find the ordinary derivative $f'(x)$ of $f(x)$ and then multiply by x defining the formal exponential derivative, with respect to x, as $xf'(x)$. We may prove easily the relation, if A and B are in $R[x]$,

$$\bar{D}_n(AB) = \sum_{i=0}^{n} \binom{n}{i} \bar{D}_i(A)\bar{D}_{n-i}(B). \tag{5}$$

We employ as a definition

$$\bar{D}\left(\frac{F}{G}\right) = \frac{G\bar{D}(F) - F\bar{D}(G)}{G^2}, \tag{6}$$

where F and G are in $R[x]$.

We now prove

THEOREM I. *Let* $\alpha = e^{2i\pi/m}$ *and*

$$f(x) = \theta_r x^r + \theta_{r-1}x^{r-1} + \ldots + \theta_0,$$

where the θ*'s are in the ring* R *of integers in* $K[\gamma, \alpha]$, *an algebraic field including* α. *Suppose that* $f(x) = 0$ *for* $x = \alpha, \alpha^2, \ldots, \alpha^{m-1}$ *and* $g(x)$ *is another polynomial in* $R[x]$, *assuming the ideal* $(g(\alpha^u), m) = (1)$ *in* R *with* u *an integer such that if* $\alpha^{uk} = 1$, *then* k *contains at least two distinct prime factors. Then*

$$\left[\bar{D}_s\left(\frac{f(x)}{g(x)}\right)\right]_{x=\alpha^u} \equiv 0 \pmod{m}. \tag{7}$$

In the above, $\bar{D}_s$ means that the fraction $f(x)/g(x)$ is differentiated s times using formal exponential differentiation. For the proof we have since $f(x)$ vanishes for $x = \alpha, \alpha^2, \ldots, \alpha^{m-1}$,

$$f(x) = \frac{x^m - 1}{x - 1} V(x),$$

with $V(x)$ in $R[x]$ and

$$\frac{f(x)}{g(x)} = \frac{x^m - 1}{x - 1} \cdot \frac{V(x)}{g(x)}. \tag{8}$$

Using equation (5) we have

$$\bar{D}_t\left(\frac{x^m - 1}{x - 1}\right) = \sum_{i=0}^{t} \bar{D}_i\left(\frac{1}{x-1}\right) \bar{D}_{t-i}(x^m - 1). \tag{9}$$

We note also that for $i < t$

$$\bar{D}_{t-i}(x^m - 1) = m^{t-i}x^m,$$

which for $x \neq \alpha^u$ reduces to an integer divisible by m, as well as

$$[x^m - 1]_{x=\alpha^u} = 0.$$

Also if we express, after employing equation (6),

$$\bar{D}_t\left(\frac{1}{x-1}\right)$$

as a simple fraction in its lowest terms, the denominator is a power of $x - 1$. When we substitute $x = \alpha^u$ in $(x - 1)^k$ we obtain a unit[4] in

$K(\gamma, \alpha)$ by the hypothesis concerning α^u. Now examine

$$\bar{D}_t\left(\frac{V(x)}{g(x)}\right)$$

and differentiate it as the product of $V(x)$ and $(g(x))^{-1}$ as in equation (9). The result may be expressed as a simple fraction whose denominator is a power of $g(x)$, and by the hypothesis concerning $g(x)$ given in our theorem the left-hand member of equation (7) may be reduced to a fraction of the form

$$\frac{\omega}{\beta},$$

with ω and β in R and $\omega \equiv 0 \pmod{m}$, $(\beta, m) = (1)$. This proves relation (7).

In applying Theorem I it is clear that $f(x)$ does not have to be given explicitly as a polynomial; we may, for example, work with $f(x)$ expressed as a product of polynomials.[5]

We do not have

$$\left[\bar{D}_t\left(\frac{x^m - 1}{x - 1}\right)\right]_{x=1} \equiv 0 \pmod{m},$$

for all values of t, but we may extend Theorem I to include $x = 1$, under a certain assumption concerning $f(1)$. For in equation (1) let, if V_1 is in $R[x]$,

$$V(x) = (x - 1)V_1 + \delta,$$

where δ is in R. Substitution in equation (1) gives

$$f(x) = (x^m - 1)V_1(x) + \delta\,\frac{x^m - 1}{x - 1}.$$

Assume $f(1) \equiv 0 \pmod{m^2}$ we obtain by setting $x = 1$,

$$f(1) \equiv 0 \equiv \delta m \pmod{m^2},$$

or $\delta = m\tau$, with τ in R. It is then easy to see that Theorem I is also true if α^u is replaced by unity if we add the assumption

$$f(1) \equiv 0 \pmod{m^2}.$$

For other cases which do not come under the assumption of Theorem I, we note that if we have $m = l$ a prime and $f(x) = 0$ for $\alpha, \alpha^2, \ldots, \alpha^{l-1}$, then

$$\frac{f(x)}{g(x)} = \frac{x^l - 1}{x - 1}\cdot\frac{V(x)}{g(x)},$$

also

$$\bar{D}_t\left(\frac{x^l-1}{x-1}\right) = x + 2^t x^2 + \ldots + (l-1)^t x^{l-1}.$$

We have the identical congruence

$$1 + x + x^2 + \ldots + x^{l-1} \equiv (x-1)^{l-1} \pmod{l},$$

whence, employing equation (5)

$$x + 2^t x^2 + \ldots + (l-1)^t x^{l-1} \equiv (x-1)^{l-t-1} h(x) \pmod{l},$$

for some $h(x)$ in $R[x]$ and this shows that for $i < l-1$ and the restriction on $g(x)$ given in Theorem I we have

$$\left[\bar{D}\left(\frac{f(x)}{g(x)}\right)\right]_{x=\alpha} \equiv 0 \pmod{\lambda^{l-i-1}}, \tag{10}$$

where $\lambda = (1-\alpha)$.

[1] Cf. for example for $n = 1$, Bachmann, P., *Die Lehre von der Kreisteilung*, ed. 2, Leipzig and Berlin, Teubner (1921), pp. 88–93, 122–150; Weber, H., *Lehrbuch der Algebra*, Braunschweig, Vieweg, (1895), ed. 1, vol. 1, 564–574, vol. 2, 632–638; for n general, Mitchell, H. H., *Trans. Am. Math. Soc.*, **17,** 167 (1916), *Ann. Math.* II, **18,** 120 (1917); Davenport, H., and Hasse, H., *J. f. Math.* (Crelle), **172,** 152–154 (1935) and other references in the Report of the Committee on Algebraic Numbers, National Research Council, *Bull. Nat. Research Council*, U. S., pt. 3, No. 28, 50–53, Washington (1923).

[2] Mitchell, H. H., *loc. cit.*, first reference.

[3] These PROCEEDINGS, **28,** 24–27 (1942).

[4] Hilbert, D., *Gesammelte Abhandlungen* Bd. 1, Berlin, Springer (1932), pp. 199, 203.

[5] If l is an odd prime dividing m then the method of introducing an indeterminate and obtaining congruences, modulo l, from relations involving exponential sums has been employed in various connections. For example, Kummer proceeded in this way in obtaining the so-called Kummer criteria for the solution of

$$x^l + y^l + z^l = 0$$

in integers (cf. Vandiver, *Ann. Math.* II, **21,** 73–78 (1919) for a proof in modern form). Further (these PROCEEDINGS, **34,** 197–203 (1948)) such devices were employed by the writer to obtain congruences involving the lth power character

$$\left(\frac{1-\alpha}{\mathfrak{p}}\right)_l \equiv (1-\alpha)^{(N(\mathfrak{p})-1)/l} \pmod{\mathfrak{p}},$$

where $\alpha = e^{2i\pi/m}$, $m \equiv 0 \pmod{l}$, $\mathfrak{p}$ is a prime ideal in $K(\alpha)$, and $N(\mathfrak{p})$ is the norm of $\mathfrak{p}$. Also the writer, *Trans. Am. Math. Soc.*, **32,** 401 (1929), lemma II, used similar methods when $m = l$ to find an explicit expression for

$$\left(\frac{1-\alpha}{\mathfrak{p}}\right)_l,$$

when $\mathfrak{p}$ is *any* prime ideal in $K(\alpha)$.

SOME RECENT APPROACHES TO THE EXPERIMENTAL STUDY OF HUMAN FATIGUE

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More than a quarter of a century ago it was proposed that the word fatigue be banished from the vocabulary of science.[8] As the title of this paper attests, this sentence of expulsion has never been enforced. The reason that the concept of fatigue retains its place in scientific writing is not hard to seek. Everyone personally feels that he knows what it is to be tired or fatigued. But in spite of this common-sense understanding, a rigorous definition acceptable to all workers in this field has never been devised. Sometimes today the word fatigue is used to apply to subjective feelings. More often it is used to describe objectively measurable changes in behavior or in underlying physiological mechanisms which follow continued activity.

Most scientists who have not themselves studied fatigue probably think of it in relation to characteristic work curves. These textbook curves ordinarily show with continued work a gradual decrement, as developed in the classical work of Mosso[7] with the ergograph. Unfortunately a transfer of the idea of this continuous work decrement to an understanding of the behavior of the total living organism in normal life situations is often most misleading.

The first of the experimental approaches to the study of fatigue in the normal adult human individual to which we wish to make brief reference in this paper is an experiment conducted by Carmichael, Dearborn, Hoffman and their associates on the continuous recording of the eye movements during long periods of continuous reading.[1]

The most important conclusion drawn from these experiments is that if subjects are sufficiently highly motivated they can continue to read with undiminished comprehension for six hours. During this whole period also there was no statistically significant change in any detail of the many movements of the eyes which are required in reading. It should be noted that while these reading experiments seemed to show clearly that 40 subjects could read for six hours without a work decrement, a less highly motivated group of subjects in a previous experiment had begun to show lapses in continuous reading in a four-hour period of work.

Other experiments may now be mentioned which were conducted several years ago under a National Defense Research Committee contract under the direction of Mead.[2, 3, 6, 11–14] The general purpose of these studies was to determine the conditions under which a decrement in be-

havior could be measured in subjects performing certain military jobs. The typical performances studied were stereoscopic ranging and eye-hand tracking. Measurements of other types of performance were also included. The usual precautions which are maintained in modern fatigue research were observed, including the conduct of preliminary practice sessions prior to the experiment proper so given as to eliminate improvement through learning.

Four subjects were required to follow a 74-hour schedule broken as follows: 42 hours on duty, which consisted of visual lookout, stereoranging or tracking; 16 hours sleeping; 8 hours eating; and 8 hours at liberty.[11] The periods during which these activities were engaged in were irregular. The stressful feature of this experiment was the irregularity and unpredictability of routines. The results showed no consistent decrement in visual lookout performance (number of target lights reported), in stereoprecision or stereoconsistency scores, or in tracking.

Despite these objective indications of lack of fatigue, the observers complained of increasing feelings of fatigue as the test progressed, particularly during the last duty period from 4:00 A. M. to 12:00 noon after $2^1/_2$ hours' sleep. Some social discord between subjects was noted.

Another experiment intended to induce a greater amount of physiological fatigue was arranged.[12] In this experiment subjects began work at 2:00 P. M. Friday and action was continued without sleep through 11:00 A. M. the following Sunday. Sets of visual ranging and tracking tests were again given intermittently during this period as in the previous test. In order to sustain interest in good stereoperformance during the trials, bonuses of 40 cents were paid on the first four trials if any precision score was better than that obtained during preliminary practice. During the subsequent nine sets of experimental trials, a bonus was paid if a score was better than that obtained during the first four experimental trials. The objective measures of fatigue were comparisons of performances during the first and the last 24 hours. Other observations and records that were taken during the course of the experiment were eye movements during ranging and tracking, accuracy and time to complete multiplication problems, reading rate and comprehension, and hand steadiness. An attempt was made to measure changes in group morale by observing behavior during card games scheduled at three stages of the three-day period. As in the previous experiments reported here, objective evidences of fatigue were negative in these tests. Stereoprecision and consistency scores during the first and last 24 hours showed no statistically significant differences. Tracking accuracy actually increased as the test progressed. This finding may perhaps be attributed to insufficient pre-test practice. Eye movement records and the speed and accuracy of multiplication showed no significant change. Hand steadiness increased during the first

28 hours and then remained constant. Reading speed showed a drop on the last two tests on Sunday morning, but reading comprehension maintained a constant level throughout. Activity, talkativeness and sociability declined during the first 24 hours. The second card game on Saturday afternoon was done quietly and without much interest. Social communication dwindled still more after this second game. A game scheduled for Sunday morning was cancelled because the subjects seemed so unresponsive. No important indications of irritability or discontent were observed. Toward the end of the experiment conversation, when it occurred, was centered on how, when and how long the subjects were going to sleep when the experiment was over. These appearances of sluggishness tended to disappear during meals and during the taking of the various tests. Again it was notable that no performance decrement was apparent in behavior which was objectively measured even after such long periods of sleep deprivation.

In another study an additional attempt was made to induce objective and measurable signs of fatigue by increasing the duress of the experimental conditions.[13] Ten male subjects were now not only required to undergo a sleep deficit but also to carry out protracted physical exercise. The test battery consisted of the following measures: (1) mean and standard deviation scores of stereorange settings, (2) differential brightness matching, (3) complex visual reaction time, (4) the average time per minute when the tracking error exceeded an arbitrary value, (5) the number of words correctly crossed out in a persistence test. This latter test consisted of crossing out all words containing both an "a" and a "t" on pages of Spanish, printed solid. There were 30 pages of Spanish in all and the subject worked for three minutes on each page for a total of 90 minutes. Bonus payments were again made for exceptional performance. A week's preliminary practice was given on all tests except the persistence test. The pre-test baseline for the persistence test occurred on the evening before the experiment proper began. The visual tests were now administered before and after three 10-mile walks so that the subjects took the tests four times and walked a total of 30 miles.

With respect to stereoscopic ranging, eye-hand tracking, differential brightness discrimination, and complex visual reaction time, no statistically significant difference between behavior when starting the tests and after sleeplessness and exercise was found. A statistically significant decrement was found in the persistence tests before and after the long hike. The performance on this test after the exercise was superior at the outset of the 90-minute work period to that found before the exercise. In other words, the subjects were physically capable of performing this monotonous task after exercise but were either unable or unwilling to exert themselves to maintain this level of performance. Toward the end of the

experiment and after the 30-mile walk the subjects almost literally "drooped" in the laboratory. Yet on the four different visual tests they pulled themselves together for the time required to make the necessary responses so that their performance was still in general as satisfactory as at first.

These experiments so far seem to suggest that a performance decrement is unlikely to be demonstrated on tasks which require discrete and short-time periods for their execution.[14] In all these experiments, including a comparison of the two reading experiments noted above, it seems that decrement in performance should be looked for with respect to an individual's interest, willingness, effort or motivation to perform a given task. If the task is continuous and of long duration, it is likely that the first existence of a physiological work decrement will be found in a decline in the subject's desire to make himself do the task especially for long-continued periods of time.

Another experiment was conducted to test the generalizations just made.[2,3] In this study a central eye-hand pursuit task had to be performed continuously while attention and occasional response had to be given simultaneously to a number of peripheral subtasks. The time for correctly performing any one of these tasks was recorded on separate clocks. Whenever either the central task or one of the subtasks was not performed adequately, the moving target would stop its motion. The total time to complete this complex task was the main criterion of performance.

Nine male college students took this test periodically throughout a three-day period in which they were deprived of sleep. Measurements of stereo-ranging were also taken at intervals during the three days. Monetary bonuses were again used to keep motivation high. The total-time scores required to complete the complex task showed a significant decrement between the first and third days. But, as expected, no deficit in stereo-ranging was found.

These results support the conclusion of the previous experiments, namely, that performance decrements due to physiological stress may be expected to occur in tasks which require continuous and prolonged effort. The fact that a decrement did occur in performing the complex task after the loss of 50 hours of sleep raises the interesting question as to whether the decline was due to actual physiological incapacity or to a breakdown of the willingness of the subjects to expend effort to keep the performance up to standard.

Because of the importance of "motivation" in these experiments and for other reasons, Kennedy, Travis and their associates developed a sensitive measure of muscle tonus which could be recorded continuously.[4, 8, 9, 10] They were interested in discovering whether or not this measure might be a valid indicator of drowsiness or loss of motivation

or lapses in alertness; that is, they were attempting to find a physiological process of which records could be made and which would parallel the fluctuations in a continuous performance, previously noted in this paper as characterizing the onset of fatigue as measured in the behavior of the total organism during long-continued periods of activity.

Muscle fibers when they contract produce characteristic potential changes. After much exploration it was found that if electrodes were placed on the skin over the frontalis muscle just above the eyebrows records could be secured that were significantly related to the alertness of the subjects studied.

A 4-channel electroencephalograph, with associated pre-amplifiers and power amplifiers, electronic counters, a voltage discriminator for counting spikes above a given amplitude, and an integrator or accumulator for continuously integrating the output from the muscle each second, was assembled.

This device has now been made in a portable form and tested in submarines, trucks and aircraft. In general, the results secured with this alertness indicator indicate that there is a definite quantitative relationship between the electric output of the chosen muscle group and the level of alertness of the subject. The highest alertness is associated with maximal muscle potential output and this output fluctuates in relation to the state of the subject until the lowest output in sleep is secured.

In conclusion then it may be said of the experiments briefly summarized here that they agree in the showing that so-called "fatigue" as it presents itself in the performance of the total normal adult human being has certain special characteristics. When the task required is not beyond the subject's normal ability, but when it is continued for a long time, the change in performance first noted is in apparently involuntary lapses in an otherwise continuous and effective performance. As such continuous and monotonous tasks go on there seems to be a conflict between motivation, or the desire to continue, and level of performance. When the subject sets himself to carry out a skilled performance after even a long period of interpolated physical work he is typically able to perform as well as in a rested state. In general, however, as time without rest progresses the number and duration of lapses increase until in a typical case sleep ensues. The pattern of human fatigue as given in these experiments is thus seen to be one of periodic blocks or interruptions in performance of varying frequency and duration rather than the continuous decrement as shown in the work curves secured in the study of isolated muscle groups.

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[12] Tufts College report to National Defense Research Comm. under Contract OEMsr-581, Report No. 4, "Effect of Sleep Deprivation Upon Performance," 1942, p. 17.

[13] Tufts College report to National Defense Research Comm. under Contract OEMsr-581, Report No. 6, "Effect of a Thirty Mile Hike on Stereoranging, Tracking, and Other Tasks, 1943, p. 30.

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*ON THE INTERPRETATION OF MULTI-HIT SURVIVAL CURVES**

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Introduction.—Survival curves, which comprise a large proportion of the data on the effects of radiation on microorganisms, generally fall into two categories: the exponential or single-hit and the sigmoid or multi-hit. It is unfortunate that in the interpretation of such data few criteria have been available other than the form of the curves themselves, but this alone has provided sufficient grounds for speculation on some properties of the organisms. Exponential survival can be explained either on the basis of a single hit to kill[1, 2] or a population distributed exponentially with respect to resistance to radiation.[3] Several considerations which make the single-

hit hypothesis seem the more plausible have been pointed out.[2, 4] Lea[5] further proposed that lethal mutation might account for the bactericidal effects of radiation, a notion which arises naturally from the finding of single-hit gene mutations in higher organisms, but which has been neither supported nor disproved by experiment. Curves of the sigmoid type may be interpreted in a similar variety of ways. If the distribution hypothesis is rejected, the number of hits, n, required to produce some criterion of inviability can be estimated from the shape of the curve. On this basis, the values of n for different types of radiation on the same material can be compared,[6] or some biological situation can be examined which may be expected to alter n.[7]

Theoretical.—Any determination of n rests ultimately upon some assumption about the way in which the sublethal hits are accumulated. The hypotheses proposed to describe multi-hit curves reduce to two which differ in this basic assumption. The first and most often used of these may be derived as follows: Let D be the average number of hits occurring within a unit volume, and let a be the sensitive volume of the organism. Then aD is the average number of hits within the sensitive volume a. If the hits occur independently and at random, the probability that X hits fall within the volume a is given by:

$$P_{(X)} = e^{-aD} \frac{(aD)^X}{X!}. \tag{1}$$

If n hits within a are required to kill an organism, then all those having less than n hits survive and the probability of survival is:

$$S = e^{-aD} \sum_{x=0}^{n-1} \frac{(aD)^X}{X!}. \tag{2}$$

The assumption implicit in equation (2) is that the presence of one or more hits within the sensitive volume has no influence on the probability of obtaining further effective hits.

Since the second multi-hit hypothesis follows directly from the single-hit hypothesis, the latter will be briefly reiterated. Let N be the number of organisms surviving a dose, D, of radiation. Let N_0 be the corresponding number at $D = 0$ and let k be a constant independent of D. Then a single-hit curve is defined by:

$$-\frac{dN}{dD} = kN \tag{3}$$

which integrated is

$$N = N_0 e^{-kD} \tag{4}$$

and the surviving fraction is

$$S = \frac{N}{N_0} = e^{-kD}. \tag{5}$$

From equation (5) it is seen that a plot of log fraction surviving against dose is a straight line of slope $-k$.[20]

Now consider a population of organisms each of which contains n units such that the units are inactivated according to equation (5) and the presence of one or more viable units insures the viability of the organism. The probability that all the units in such a group of n become inactivated is:

$$P_{(n)} = (1 - e^{-kD})^n. \tag{6}$$

Then the proportion of organisms surviving is:

$$S = 1 - (1 - e^{-kD})^n. \tag{7}$$

The assumption here is not simply that n hits per organism are required, but that each of n particles within the organism must be hit at least once. It is evident that equation (7) may also be derived as a special case of equation (2).[8] Expressions (2) and (7) describe curves of the same general form, and for low dose and high values of n where terms of the order of $(kD)^n/n!$ can be ignored, they become identical. It is impossible in most cases to decide from experimental data which equation should be used as a working model. Nevertheless, the choice is important because it is possible for numerical values of n obtained from one experiment to differ by an order of magnitude, depending upon which hypothesis is chosen. As will be seen later in this paper, there are additional criteria which point to expression (7), or some development thereof to cover additional assumptions, as the more reasonable in the interpretation of multi-hit curves.

The use of equation (2) and its extension to more complex cases has been discussed at length elsewhere,[9, 10] hence we confine ourselves to exploring some features of equation (7).

Expanding equation (7):

$$S = 1 - (1 - ne^{-kD} + \ldots \pm e^{-nkD}). \tag{8}$$

It is seen that as D increases, the terms containing e^{-2kD}, e^{-3kD}, ..., etc., become negligible in comparison to ne^{-kD}. Thus, at high dose:

$$\log S = \log n - kD. \tag{9}$$

Extrapolating equation (9) to $D = 0$ gives n (Fig. 1). Having obtained values for n and k we should be able to reconstruct the convex portion of the curve from equation (7) and show that the reconstruction fits the experi-

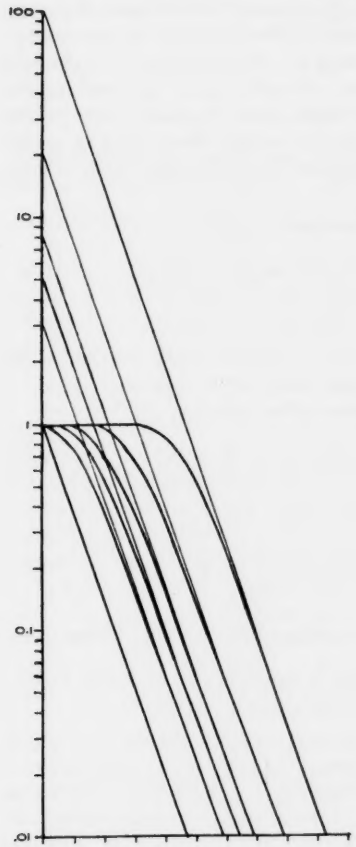

FIGURE 1

Ordinate, survival. Abscissa, dose in arbitrary units. Theoretical curves for $S = 1 - (1 - e^{-kD})^n$, $k = 1$. From left to right n is 1, 3, 5, 8, 20 and 100. Linear portions of the curves are extrapolated to $D = 0$.

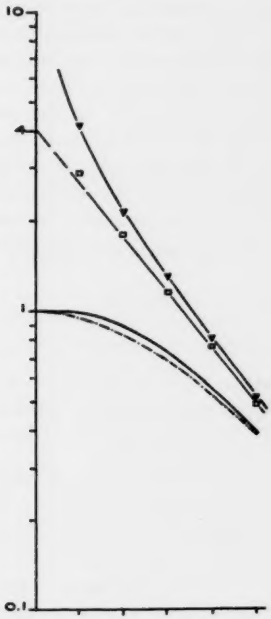

FIGURE 2

Ordinate, S for lower curves, g for upper curves. Abscissa, dose in arbitrary units. Lower curves: Solid line is theoretical curve for $S = 1 - (1 - e^{-kD})^n$ where $n = 4$ and $k = 0.43$. Broken line same, but n has the distribution: 7% 1, 15% 2, 20% 3, 21% 4, 16% 5, 11% 6, 7% 7 and 3% 8, which averages 4. Upper curves: Triangles are points computed from solid line curve by formula: $g = -\ln (1 - S)$. Squares are points similarly computed from broken line curve. The g plot for distributed n is linear at higher survival than for constant n.

mental data in this region if equation (7) holds. However, while the basic assumption from which equation (7) is derived may hold, equation (7) may not be sufficient to describe experimental data because of at least two additional eventualities which seem, *a priori*, likely to be encountered. First, n may not be a fixed number when $D = 0$, but instead n might have some distribution in the population. Second, more than one process might be simultaneously occurring within each organism, each process having its own value of n and k. In other words, there might be several classes of sensitive units with the presence of at least one viable member of each class necessary for survival.

In the case of a population distributed for n, equation (7) becomes:

$$S = 1 - [C_1(1 - e^{-kD})^{n_1} + C_2(1 - e^{-kD})^{n_2} + \ldots + C_l(1 - e^{-kD})^{n_l}], \tag{10}$$

where $C_1 + C_2 + \ldots + C_l = 1$ and C_i represents the fraction of the population having n_i units. For high dose, as in equation (8), $(1 - e^{-kD})^n \backsimeq 1 - ne^{-kD}$. With this substitution, equation (10) becomes:

$$S = e^{-kD}(C_1n_1 + C_2n_2 + \ldots + C_ln_l). \tag{11}$$

Thus:

$$\log S = -kD + \log \sum_{i=1}^{l} C_in_i = \log \bar{n} - kD \tag{12}$$

where $\bar{n} \equiv \sum_{i=1}^{l} C_in_i$ is the ordinary arithmetical average. Thus extrapolation to $D = 0$ of the linear portion of equation (10) on a semi-log plot gives the average value of n for the distribution present at $D = 0$.

The shape of the curve will be changed according to the type of distribution, since the presence of fractions of the population having values of n below the average value will cause the curve to fall off more rapidly than it would when n is a constant (Fig. 2, lower curves). Such changes are difficult to interpret by inspection or curve fitting, but can be perceived more easily from a semi-log plot of $-\ln(1 - S)$ against dose (Fig. 2, upper curves). The use of this device is justified as follows:

From equation (5), let $p = e^{-kD}$ be the probability that a unit (within an organism) survives. Let P be the probability that m units survive within an organism containing a total of n units. Then:

$$P_{(m=i)} = \binom{n}{i} p^i (1 - p)^{n-i} \tag{13}$$

As D becomes large, p approaches 0 and,

$$P_{(m=i)} \backsimeq e^{-g}\frac{g^i}{i!} \tag{14}$$

where

$$g = np = ne^{-kD}. \tag{15}$$

The probability that an organism is killed is the probability that no units survive, and where equation (14) holds is:

$$P_{(m=0)} = e^{-g} = e^{-ne^{-kD}}. \tag{16}$$

Thus e^{-g} is equated to the fraction of organisms killed, and from experimental data and equation (16) we can solve for g, which is the expected number of surviving units per organism. Then, from equation (15), when D is large:

$$\log g = \log n - kD. \tag{17}$$

Again extrapolating to $D = 0$ we reach $\log n$. Note that equation (17) has the same form as equation (9), and that the extrapolated line in both cases represents the theoretical survival curve of the units as a function of dose. But $\log g$ will have a linear relation to dose wherever approximation (14) holds, and expression (14) states that the surviving units have a Poisson distribution among the organisms. Thus, if the initial distribution of n (where $D = 0$) resembles more or less a Poisson distribution, then $\log g$ versus D will continue to be more or less linear into the low dose range. If, at $D = 0$, n is a constant as in equation (7), $\log g$ will have spurious values at low doses (Fig. 2, top curve). Intermediate initial distributions will give values of g between these two extremes.

Now suppose that there are two classes of sensitive units with l of one sort and n of another within the same organism, and that the inactivation of all of either class kills the organism. Let $P(A)$ be the probability that an organism survives process A affecting the class of units of number l and $P(B)$ be the probability that an organism survives process B affecting the class of units of number n. Then the probability that an organism survives is the probability that it survives both processes,

$$P(AB) = P(A)P(B), \tag{18}$$

if the two processes are independent. From equation (16):

$$P(A) = 1 - e^{-g_1} = 1 - e^{-le^{-k_1D}} \tag{19}$$

and,

$$P(B) = 1 - e^{-g_2} = 1 - e^{-ne^{-k_2D}}.$$

Thus,

$$P(AB) = (1 - e^{-g_1})(1 - e^{-g_2}) = 1 - e^{-g_1} - e^{-g_2} + e^{-(g_1 + g_2)} \tag{20}$$

If $n \gg l$, then at low dose $P(B) \backsimeq 1$ and $P(AB) \backsimeq P(A)$, and $P(A)$ will approximate $1 - e^{-g_1}$ more or less well, depending on the initial distribution of the units of average number l, and on the dose reached before $P(B)$ becomes noticeably less than 1. When a sufficiently high dose is reached, expression (20) holds.

Where $S = P(AB)$ is the survival of both processes, and $g \equiv -\ln(1 - S)$, the plot of log g versus D forms two lines (Fig. 3, middle curve). The first line represents the process prevailing in the low dose region, approaches a slope of $-k_1$, and if this slope is reached, extrapolates to l at $D = 0$. The second line represents the combined processes, approaches a slope of $-(k_1 + k_2)$ and extrapolates to ln at $D = 0$. The extension to more than two processes is obvious. It is also evident that in practice, the possibility of inferring two or more processes is dependent on the relative values of n, l, k_1, k_2, etc. The presence of an inflection point in the g curve (as in Fig. 3, lower curve) will, however, in general indicate more than one process even though the process affecting the class of units of lowest number may not have proceeded far enough to validate equation (17) before it is masked by the next process.

Let us now consider the multi-process case likely to be encountered if the effect of radiation is predominantly on the genetic apparatus of organisms in which the genetic material is present in more than one set. Let n be the number of sets of genes, each set consisting of m genes, or each set being of such length as to include m deficiencies of average length. From equations (18) and (7):

$$S = \prod_{i=1}^{m} [1 - (1 - e^{-kD})^n] \tag{21}$$

In particular, where $k_1 = k_2 = \ldots = k_m = k$, i.e., where all genes or regions have the same sensitivity:

$$S = [1 - (1 - e^{-kD})^n]^m \tag{22}$$

At high dose, by the usual approximation,

$$S \backsimeq n^m e^{-mkD} \tag{23}$$

and extrapolation to $D = 0$ of the linear portion of log S, whose slope is $-mk$, gives the value log $(n)^m$.

The above may be summarized as follows:

(*a*) For curves of the form of equations (7) or (10) a semi-log plot of S versus D is a curve approaching a line of slope $-k$ as D increases.

(*b*) Extrapolation of the linear portion of such a curve to $D = 0$ gives n.

(*c*) If at $D = 0$, n has a distribution, then extrapolation as above gives the average value of n.

(*d*) The shape of the curve is altered by such a distribution and also by the eventuality that killing by two or more independent processes occurs.

(*e*) A multi-process effect changes the slope of the linear portion of the curve and changes the meaning of its intercept at $D = 0$. The presence of these complications is more evident on inspection if $g \equiv -\ln(1 - S)$ is plotted instead of S.[11]

Experimental.—To show how the theory yields a reasonable result when applied to experimental data and how the g plot facilitates interpretation some data on ultra-violet irradiation of *Neurospora crassa* macroconidia will be presented (to be reported in greater detail elsewhere). Since the macroconidia are known to be multi-nucleate, equation (21) will hold at least with respect to processes occurring within the nuclei.

The ultra-violet source was a 30-inch Westinghouse sterilamp low-pressure mercury vapor tube stated to deliver 85 per cent of its energy in the 2537 Å. region. Suspensions containing ca. 200,000 conidia per

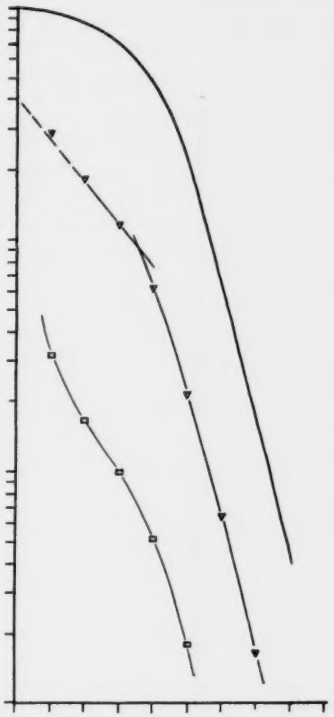

FIGURE 3

Ordinate, S for upper curve, g for lower curves. Middle curve has been moved down one decade and lowest curve two decade. Abscissa, dose in arbitrary units. Upper curve: Theoretical curve for simultaneous survival of two processes, one with $k = 0.43$ and the distribution of n given in Fig. 2, and the other having $k = 1$ and $n = 100$. Middle curve: g computed from upper curve. Lower curve: g computed from theoretical survival of two processes, one having $k = 0.43$ and $n = 3$ (constant), and the other having $k = 1$ and $n = 100$.

cubic centimeter in 0.9 per cent saline were exposed in an open petri plate at a distance of 15 cm. from the center of the tube. The plate was tilted at an angle of 15° from the horizontal and slowly rotated throughout exposure. The average depth of suspension was 5 mm.

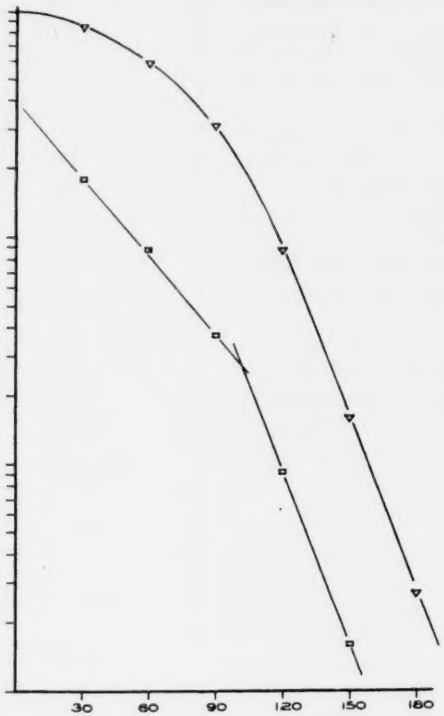

FIGURE 4

Ordinate, *S* for upper curve, *g* for lower curve. Lower curve moved down one decade. Abscissa, seconds of exposure to ultra-violet light under conditions given in text. Upper curve: Survival of macroconidia of *Neurospora crassa* heterokaryon (1633–422)A-(5531–4637)A. Lower curve: *g*, computed from experimental points.

The strain used was a heterokaryon having as one component the double mutant aminobenzoicless-morphological, (1633-422)A, and the other the double mutant pantothenicless-albino (5531-4637)A. This gives normal growth and produces macroconidia on minimal medium. The criterion

of viability was the ability to form a visible colony in a sorbose medium.[12] Plates were incubated at 37° in darkness for five days. All handling of irradiated suspensions was done by the light of red photographic safelights to prevent photoreactivation.[13]

Table 1 shows the survival relative to exposure time in seconds and the corresponding values of g. Figure 4 shows the semi-log plot of S and g.

We note from the g plot in figure 4 that the conidia seem to be killed by more than one process, the low dose portion having an intercept at 4. Subsequently, the intercept assumes a minimum value of ca. 130. Further, since the values of g form nearly a straight line at low dose, we conclude that 4 is an average value for the number of some sensitive unit present in the conidia at $D = 0$.

Figure 5 shows the distribution of nuclei in the conidia of the stock used in this experiment, as determined by counts done on preparations fixed and stained by a modification of Robinow's technique.[14] The average is 2.67 nuclei per conidium. If the killing at low dose is related to the number of nuclei present, then 1.5 hits per nucleus are required to agree

TABLE I

THE SURVIVAL OF MACROCONIDIA EXPOSED TO ULTRA-VIOLET LIGHT

Time, seconds	S	1-S	g
30	0.84	0.16	1.8
60	0.59	0.41	0.89
90	0.31	0.69	0.37
120	0.088	0.912	0.092
150	0.016	0.984	0.016
180	0.0027	0.9973	0.0027

with $n = 4$. Independent findings of Goodgal[15] and Giles[16] using microconidia known to be uninucleate show that these have a 1.5 hit curve with ultra-violet. Thus, it seems probable that the 4-hit component found here is related to the number of nuclei in the macroconidia.

This relationship must mean that there is some region in each nucleus which is especially sensitive and is responsible for practically all the effect at low dose. If the entire curve is interpreted according to equation (21) we would conclude that at higher doses additional less sensitive regions begin to contribute to the effect. An alternative interpretation based on equation (20) is that there is a very sensitive region as above, but that there is another single independent process involving ca. 30 units of greater sensitivity, and not necessarily related to the nuclei. Obviously, further criteria are necessary to distinguish between these possibilities. The reason for the finding of a 1.5-hit curve for uninucleate conidia is not yet clear.

Discussion.—The reasons for preferring the single-unit action interpretation of exponential curves have been discussed by Lea, Haines and

Coulson.[2] Since the theory presented here is based on the assumption that organisms contain units which themselves are inactivated exponentially, the arguments advanced by Lea, *et al.*, in favor of the single-unit action interpretation are again applicable. If we accept these, then the question of whether the theory is reasonable will depend on our reasons for believing that microorganisms showing multi-hit survival contain a number of discrete units rather than a target requiring a certain number of hits.

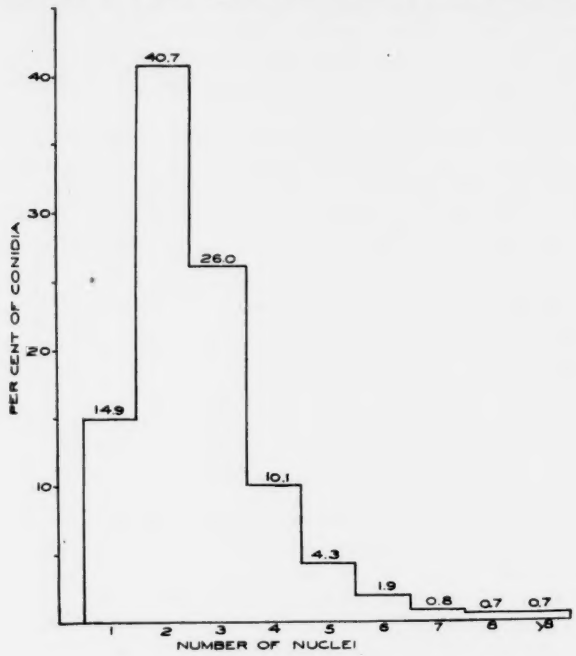

FIGURE 5

Distribution of nuclei in conidia of stock used in Fig. 4; 2134 conidia were scored.

In the special cases we have found where equation (7) has been used previously, the reasons for preferring it to equation (2) are obvious: One of these cases is an explanation of spurious multi-hit curves due to clumping of bacteria[2] and the other is the experiment of Luria and Latarjet designed to follow the increase in numbers of intracellular bacteriophage by means of the survival curves of the infective centers.[7] Equation (22) has recently been given by Luria and Dulbecco[17] in the estimation

of the number of genetic units in a phage particle. In other cases where values of n are reported (see review by Zimmer[18]), they have apparently been obtained by fitting the experimental data to theoretical curves prepared from equation (2).

A strong argument for the more general use of equation (21) as a working hypothesis is the repeated demonstration in various microorganisms of genetic mechanisms comparable to those in higher organisms. It is evident that organisms in which the genetic material is duplicated in some way, as by diploidy, polyploidy or multi-nucleatedness, may be expected to give multi-hit curves, and these should be of type (21), but not type (2), in so far as the effects of radiation on the genetic apparatus affect viability. In general, where lethality is due to the exhaustive inactivation of groups of identical essential parts, equation (2) must be rejected.

In the case of a large number, m, of identical units any $n \ll m$ of which are indispensible, the probability of obtaining an effective hit would not be decreased significantly even when $n - 1$ of the units had already been altered. This satisfies the requirements of equation (2). However, it seems unlikely that an organism could be killed by a process which is so far from exhaustive.

There are further considerations which make equation (2) seem untenable even when biological criteria are lacking. This becomes clear when we try to think of the observed biological effects in terms of underlying molecular transformations. Assume that a dose, D, has a probability, p, of changing one of n molecules. Subsequently, an identical dose will have a probability less than p of causing the same change in one of the $n - 1$ remaining unaffected molecules. This is true regardless of whether the transformation of the molecule results from the absorption of a quantum directly (since the probability of such an absorption is proportional to the number of molecules present) or whether the transformation is the end result of a series of chemical reactions, since the probability of occurrence of the reaction which involves the essential molecule is also proportional to the number of such molecules present.

This situation is contrary to the assumption underlying equation (2). In attempting to construct models consistent with the assumptions underlying equation (2) we encounter serious difficulties. For example, a model based on a molecule requiring the absorption of n quanta before it is transformed is objectionable because it implies an unheard of stability of the intermediate excited states, and also leads to intensity dependence. In short, the existence of such a molecule seems contrary to established photochemical principles.

It should be emphasized that equation (7) does not imply any necessity for regarding the units as targets upon which a hit must be scored directly, since the units themselves would show exponential survival regardless of

whether the action is direct or indirect.[5] Thus, with radiations giving different ion-densities any differences in the survival curves which are found to be inconsistent with target geometry do not invalidate equation (7) or its corollaries as a model for multi-hit curves. The finding of lowered values of n with radiations of high ion density[19] is consistent with the theory whether or not there is significant indirect effect. With direct effect, this would be due to the increased probability of an ionizing particle traversing more than one unit, and since ionizations produced between the units would be ineffective, there would be a concomitant decrease in k. With indirect effect, n would also decrease with increasing ion density if each ionizing particle produced sufficient ionizations to inactivate more than one unit, but here no change in k would be expected since each ionization would have an equal chance of being effective. In situations described by equation (21) an increase in m might be found with high energy radiations due to the equalizing of sensitivity between the various regions or genes involved. As an isolated effect this would increase the over-all value of k. However, as we can hardly expect any of these effects to be observed in pure form, it seems most reasonable in comparing multi-hit curves with different types of radiation to consider the differences in terms of a balance between the various factors tending to shift the parameters n, m and k.

Summary.—It is proposed that sigmoid survival curves can be interpreted profitably on a multi-unit, single hit per unit hypothesis, and some consequences of this hypothesis are examined. An aid to the analysis of experimental data is given and its use is illustrated. Reasons are given for preferring the multi-unit hypothesis to the usual multi-hit hypothesis. Some data on the ultra-violet irradiation of *Neurospora crassa* conidia are analyzed.

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ATYPICAL PIGMENT-CELL DIFFERENTIATION IN EMBRYONIC TELEOSTEAN GRAFTS AND ISOLATES

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The occasional occurrence of red blood corpuscles and of chromatophores in otherwise non-differentiating isolates and grafts from teleostean gastrulae has been casually mentioned in an earlier publication[1] concerned with other problems than pigment-cell formation. In view of the increasing interest in pigment-cell formation since that time, ensuing upon DuShane's[2] establishment of the neural crest origin of the melanophores in the amphibian, it now seems relevant to present some of the data concerning the atypical differentiation of teleostean chromatophores.

Fundulus Germ-Ring Grafts.—In this series of experiments, portions of the germ-ring located 90° or 180° from the midline of the embryonic shield of *Fundulus heteroclitus* gastrulae (cf. Fig. 1 *B*, Oppenheimer[1]) were grafted to the embryonic shield or to the extra-embryonic membrane of gastrulae of the same species. In thirty-seven cases either the whole grafts or portions of them continued development but failed to undergo typical histogenesis and differentiated no axial structures. In sixteen of these, however, red blood corpuscles differentiated, and in ten of these sixteen grafts melanophores differentiated. The ten grafts differentiating melanophores included five implanted on yolk-sac epithelium, and three which

were implanted onto the embryonic shield but which, in whole or in part, remained external to the host epithelium and failed to differentiate typically. In the remaining two cases parts of the grafts were incorporated into the host embryos and differentiated axial structures, but adjacent portions of them remained undifferentiated.

In some cases, only a single melanophore was differentiated within the grafts, in others a larger number was present. Only those grafts are included among the ten positive cases enumerated above which contained melanophores located more or less centrally in the mass of cells; grafts to which melanophores were applied only at the surface are excluded from consideration in the possibility that in these cases host melanophores might have migrated to cover the grafts.

In the absence of heteroplastic grafting, there is indirect evidence only that the melanophores were derived from graft rather than host cells. In several cases the melanophores were considerably more densely packed in the grafts than in the adjacent host tissues, and there is no particular reason to suppose that the grafts should attract chromatophores from the host tissues in such numbers as to trap them in higher concentration than usual. In some cases unbroken host epithelium separated graft and host tissues, presumably presenting a barrier to migration. The graft melanophores were occasionally intermingled with the masses of red blood corpuscles which are known to have developed *in situ* since connecting blood vessels between graft and host were absent.

Only heteroplastic transplantation or the grafting of materials from vitally stained embryos can furnish direct proof that the melanophores are formed by graft rather than host cells in such experiments as these. Fortunately, however, supplementary data are available from other types of experiment which demonstrate that chromatophores are also similarly differentiated in some cases by isolates rather than transplants from teleostean gastrulae.

Epiplatys Germ-Ring Isolates.—The embryos of *Epiplatys fasciolatus* can be divided at late gastrula stages in such a way (cf. Fig. 1 *A*, Oppenheimer[1]) that the portion of the germ-ring most remote from the embryonic shield can be isolated with part of the yolk from the portion of the egg containing the shield. Such isolates develop for several days in Ringer's solution. Though in some cases they exhibit certain phenomena of growth which simulate the form of the tail, they undergo no histogenesis of axial structures. Of twelve such aggregates studied, one developed red blood corpuscles, one formed a single melanophore and a third differentiated a number of xanthophores. One explant of prospective tailbud region, isolated immediately after the closure of the blastopore, formed both melanophores and xanthophores although otherwise undifferentiated.

Fundulus Isolated Blastoderms.—Blastoderms of *Fundulus heteroclitus*

separated from the yolk and cultivated in double-strength Holtfreter's solution under some conditions form hyperblastulae, masses of non-differentiated cells provided with a large vesicle at one pole.[3] Of approximately seventy-five hyperblastulae studied, one only developed a single melanophore of typical size and configuration.

Discussion.—The production of chromatophores by the grafts and isolates described above suggests that in the teleost under certain experimental conditions pigment-cells can be differentiated by cells which normally do not contribute to the teleostean counterpart of the neural crest. The 90° germ-ring of *Fundulus* normally contributes primarily mesoderm to the embryo;[4] and the 180° germ-ring is unrelated to nervous system formation except in so far as it contributes to the tailbud blastema from which neural derivatives later may arise. While it is theoretically possible, it is highly improbable that the chromatophores in these cases have been differentiated only by the particular cells destined to form them later after their passage through the tailbud blastema. The differentiation of pigment-cells under these conditions is therefore presumably the result of what the experimental embryologists call "bedeutungsfremde Selbstdifferenzierung."

This interpretation, however, is not incompatible with the possibility that in teleosts the chromatophores normally arise from cells corresponding to those of the neural crest of other vertebrates, as suggested by the results of the transplantation experiments of Lopashov[5] on three species of teleosts, and in line with the neural crest origin of pigment-cells in amphibians,[2] birds[6] and mammals.[7] It merely signifies that under certain abnormal conditions, other than the usual cells can take over the function of pigment-cell formation.

The differentiation of red blood corpuscles in the same series of preparations, and in some cases by the same grafts that differentiated the chromatophores, suggests that the production of these cell types is the result of a generalized type of reaction. Somewhat comparable differentiation of red blood corpuscles in otherwise non-differentiating grafts has been described for the chick;[8] and somewhat analogous to this condition may be the differentiation in chick embryonic grafts of ganglion cells formed in the absence of differentiation of central nervous tissue.[9]

Whatever the factors may be in these grafts and isolates that suppress their typical differentiation or that fail to support it, they seem to be allied with factors that foster the atypical production of chromatophores by cells originally destined to fulfil other functions. The fact that such conditions obtain is of twofold significance. In the first place, they suggest that in the teleosts at least, great caution must be exercised in the interpretation of transplantation- or explantation-experiments designed to elucidate the normal development of pigment-cells. More positively,

they offer the hope that since in these forms pigment-cells can be produced under experimental conditions by other than typically differentiating cells, it may prove experimentally feasible in them to analyze some of the factors responsible for this abnormal differentiation, and hence to shed further light on the normal mechanism of production of these cells in the typically differentiating embryo.

Summary.—Grafts of germ-ring of *Fundulus heteroclitus* gastrulae, exhibiting no other typical histogenesis, have in some cases been found to differentiate red blood corpuscles and melanophores. Red blood cells, melanophores and occasionally xanthophores have been found present in tail-shaped structures lacking other histological differentiation formed by portions of *Epiplatys fasciolatus* germ ring isolated in Ringer's solution. In a single case out of approximately seventy-five studied, a melanophore was differentiated in the absence of other histogenesis by a hyperblastula formed by an early *Fundulus heteroclitus* blastoderm removed from the yolk and cultivated in double-strength Holtfreter's solution. These facts in no way negate the possibility that in teleosts chromatophores are normally provided by cells comparable to the neural crest of amphibians, birds and mammals. They suggest, however, that under certain experimental conditions not adequate to foster normal histogenesis, cells other than those destined for such differentiation are capable of forming pigment-cells.

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ERRATA

In the article "Studies on the Biochemical Genetics of Yeast," these PROCEEDINGS, pages 456–464, August, 1949, the reference[21] on p. 459, 8th line up, and on p. 460, 5th line down, should be [20a], and among the references on p. 464 should be inserted:

[20a] Burkholder, P. R., and Giles, N. H., Jr., *Am. J. Botany*, **34**, 345–348 (1947). SEYMOUR POMPER

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